# Biochemical studies on some species of MORINGA

## In North Africa

2015

Mahmoud Abdel-Ghany
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# BIOCHEMICAL STUDIES ON SOME SPECIES OF *MORINGA* IN NORTH AFRICA

***By***

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**B.Sc. Agric. Sci. (Agricultural Production), Fac. Agric., Cairo Univ., 1996**

**Diploma of Applied Biochemistry (Biochemistry), Fac. Agric., Cairo Univ., 2004**

**M.Sc. of Biochemistry (Biochemistry), Fac. Agric., Cairo Univ., 2011**

## THESIS

**Submitted in Partial Fulfillment of the**

**Requirements for the Degree of**

## DOCTOR OF PHILOSOPHY

*In*

## AFRICAN STUDIES

**(***Natural Resources-Plant Resources***)**

**"Biochemistry"**

**Department of Natural Resources**

**Institute of African Research and Studies**

**Cairo University**

**EGYPT**

**2015**

## APPROVAL SHEET

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Date: 14 / 12 / 2015

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Date: 14 / 12 / 2015

SUPERVISION SHEET

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## ABSTRACT

The aim of this investigation were to determine and evaluate (*in vitro*) the chemical composition of leaves and seeds contents for *Moringa oleifera* (MO), *Moringa stenopetala* (MS), *Moringa peregrina* (MP) which were collected from Egypt. The results of the chemical composition analysis showed that *Moringa* species leaves and seeds have high significant nutritional values. Moisture, ash, protein, fat, fiber, carbohydrate, energy, elements; Ca, Mg, Zn, Cu, vitamins C and A, amino acids compositions, total phenolic contents and antioxidant activities against DPPH free radical were determined. Meanwhile, the oil proprieties evaluated and the results showed significant decreases in; free fatty acids percentage's, peroxide values, iodine values and showed significant increases for saponification values. Also, the fatty acids compositions were evaluated and the major saturated fatty acids; palmitic acid (C16:0), stearic (C18:0) and behenic (C22:0) recorded high significant values. On the other hand, the main unsaturated fatty acids, oleic acid (C18:1n9); eicosenoic (C20:1n9) and palmitoleic acids (C16:1n7) recorded significant values.

In addition to evaluate (*in vivo*) the effects of ethanolic and aqueous extract of these species leaves on streptozotocin (STZ) induced diabetes rats.

Fasting (FBG) and postprandial blood glucose (PBG) levels, ALT, AST, Creatinine, Triglyceride (TG), Total Cholesterol (TC), LDL and HDL were evaluated. Significant reduction $p<0.05$ was recorded in glucose levels of the diabetic rats compared with diabetic control group expressing their hypoglycaemic potentials. PBG, Creatinine, TC, HDL, LDL levels, ALT and AST activity showed significant decreasing. On the other hand, TC and TG level showed significant decrease compared with diabetic control group. Meanwhile, hisopathological examination for liver, kidney and pancreas tissues showed more improving for group treated with *Moringa oleifera* than the other groups. That balances and different may referred to many factors; environments, climate, origin, genetic, fertilization, temperature range and flowering and yielding time.

Generally, conclusion use of natural antidiabetic of *Moringa oleifera* leaves might be useful for decreasing PBG level and the effects against pancreatic damage, moreover, improves liver and kidney functions.

**Key words:** *M*oringa *olifera; Moringa stenopetala; Moringa peregrina*; Fatty Acids ; STZ; Antidiabetes; Antioxidant; Total Phenolic Contents; DPPH.

# *DEDICATION*

*I dedicate this work to whom my heartfelt thanks; to my parent's spirit for all the support they lovely offered along the period of my post graduation, as well as to my wife Halah and my kids Ezz-Eldeen, Rawan, Omar and Abdullah for their patience and help.*

## *ACKNOWLEDGEMENT*

*First and Foremost, All praise and thanks are to Allah, The magnificent, the merciful, without whose bless and guidance this work would never have been started nor completed.*

*I wish to express my sincere thanks, deepest gratitude and appreciation to* ***Prof. Dr. Fouad A. Ahmed*** *Professor of Biochemistry, Faculty of Agriculture, Cairo University,* ***Dr. Shereen A. Nada,*** *Professor of nutrition , Regional Center for Food and Feed, Agric., Research Center, Giza,* ***Dr. Amira Shawky Soliman*** *and* ***Dr. Mohamed Said Abbas,*** *Assistant Professors of Plant Resources, Department of Natural Resources, Institute of African Research and Studies, Cairo University, for suggesting the problems, supervision, continued assistance and their guidance through the course of study and revision the manuscript of this thesis. Sincere thanks to* ***Dr. Youssef F. Ahmed,*** *Professor of Animal Pathology, Dept. of Animal Reproduction and A.I, National Research Center for suggesting the problems, supervision and continued assistance.*

*Special thanks are directed to* ***Dr. Abdel-Rahman M. Ahmed*** *Professor of Citrus, Citrus Research Department, Horticulture Research Institute, Research Center, Giza, Egypt.*

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

| Abb. | Full form |
|---|---|
| AA | L-Ascorbic acid |
| $ABTS^{\cdot}$ | 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate) |
| ACP | Acid phosphatase |
| AIN | American Institute of Nutrition |
| ALP | Alkaline phosphatases |
| ALT | Alanine aminotransferase |
| AOA | Antioxidant activities |
| $Aq^{\cdot}$ | aqueous radical |
| AqH | aqueous hydrogen donor |
| ARC | Agriculture Research Center |
| $Asc^{\cdot}$ | ascorbyl radical |
| AscH | ascorbic acid |
| AST | Aspartate aminotransferase |
| b.w | Body weight |
| BALB | an albino strain of laboratory mouse |

| | |
|---|---|
| BHA | Butylated hydroxyl anisole |
| BHT | Butylated hydroxyl toluene |
| β-GAL | β-Galactosidase |
| β-NAG | N-acetyl-B-glucosaminidase |
| CAR | carotenoid |
| CAR+ | carotenoid radical cation |
| CAT | Catalase |
| $CCl_3^{\cdot}$ | Trichloromethyl free radicals |
| $CCl_3OH$ | Trichloromethanol |
| $CCl_4$ | Carbon tetrachloride |
| CDNB | 1-chloro-2,4-dinitrobenzene |
| CE | Catechin equivalents |
| $CHCl_3$ | Chloroform |
| CPR | Cytochrome P450 Reductase |
| CVD | Cardiovascular disease |
| CYP | Cytochrome P450 monooxygenase |
| DEN | N-diethylnitrosamine |

| | |
|---|---|
| Didox | 3,4-dihydroxybenzohydroxamic acid |
| DMBA | 7,12-dimethylbenz[a]anthracene |
| DNA | Deoxyribonucleic acid |
| dNTPs | Deoxynucleoside triphosphate pools |
| DPPH· | l,l-diphenyl-2-picrylhydrazyl radical |
| DTNB | 5-5'-dithiobis [2-nitrobenzoic acid] |
| EA | Ellagic acid |
| EC | Enzyme Code |
| ECG | (-)-Epicatechin-3-gallate |
| EGCG | Epigallocatechin-3- gallate |
| EGCG-O· | (-) Epigallocatechin gallate radical |
| EGCG-OH | (-)-Epigallocatechin gallate |
| ESC | Experimental Scavenging Capacity |
| ESR | Electronic spin resonance |
| Fe-NTA | Ferric nitrilotriacetate |
| FID | Flame Ionization Detector |
| FR | Free radicals |

| | |
|---|---|
| FRAP | Ferric reducing antioxidant power |
| GA | Gallic acid (3,4,5-trihydroxybenzoic acid) |
| GAE | Gallic acid equivalent |
| GC | Gas chromatography |
| GC-MS | Gas Chromatoghraphy-Mass Spectrometry analysis |
| GGT | γ-glutamyl transferase |
| GJE | *G. jasminoides* Ellis |
| GLC | Gas liquid chromatography |
| GOT | Glutamic-oxaloacetic transaminase |
| GPT | Glutamic-pyruvic transaminase |
| GPx | Glutathione peroxidase |
| GRd | Glutathione reductase |
| GSH | Reduced glutathione |
| GST | Gutathione-S-transferase |
| GTPs | Green tea polyphenols |
| H | Hour (s) |
| $H_2O_2$ | Hydrogen peroxide |

| | |
|---|---|
| $HepG_2$ | Hepatoblastoma $G_2$ |
| HL-60 | Human Leukemia |
| HOCl | Hypochlorous acid, HL-60 cells |
| HPLC | High performance liquid chromatography |
| IDF | International Diabetes Federation |
| i.p | Intraperitoneal |
| IL-2 | Interleukin-2 |
| IL-4 | Interleukin-4 |
| IR | Irradiation |
| L | Lipoxygenase |
| $LD_{50}$ | Lethal dose |
| LDH | Lactate dehydrogenase |
| LDL | Low-density lipoprotein |
| LPx | Lipid peroxidation |
| LSD | Lowest significant differences |
| MDA | Malondialdehyde |
| MED | Medicine |

| | |
|---|---|
| MFOS | Mixed function oxidase system |
| MO | *Moringa oleifera* |
| MON | *Moringa oleifera* from Nubarya |
| MOS | *Moringa oleifera* from Shalateen |
| MP | *Moringa peregrina* |
| MPFI | Moringaling Philipines Foundation Inc. |
| MPG | *Moringa peregrina* from Giza |
| MS | *Moringa stenopetala* |
| Ms | Mass spectrometry |
| MSA | *Moringa stenopetala* from Aswan |
| MSB | *Moringa stenopetala* from Belbis |
| NAD | Nicotinamide adenine dinucleotide |
| NADP | Nicotinamide adenine dinucleotide phosphate (oxid.) |
| NADPH | Nicotinamide adenine dinucleotide phosphate (red.) |
| NF-B | Nuclear Factor-B cells |
| NFE | Nitrogen-Free Extract |
| NFF | Nutraceuticals and functional foods |

| | |
|---|---|
| NHP | Natural Health Products |
| NIDDM | noninsulin-dependent diabetes mellitus |
| $NO^{\cdot}$ | Nitric Oxide |
| $^{1}O_2$ | Singlet Oxygen |
| $O^{\cdot}{}_2$ | Superoxide radicals |
| $O^{\cdot}{}_2^{-}$ | Superoxide anion |
| $OH^{\cdot}$ | Hydroxyl radical |
| $ONOO^{-}$ | Peroxynitrite |
| $OOCCl_3$ | Proxy trichloromethyl radical |
| ORAC | Oxygen radical absorption capacity |
| PAL | Phenylalanine ammonia lyase |
| PCA | Protocatechuic acid |
| PKC | Protein kinase C |
| PP | Polyphenols |
| Pr-SHs | Protein thiols |
| PUFA | Polyunsaturated fatty acid |
| $R^{\cdot}$ | Formation alkoxy |

| | |
|---|---|
| r.p.m. | Rotary per minute |
| RNA | Ribonucleic acid |
| RNS | Reactive nitrogen species |
| $ROO^{\cdot}$ | Peroxy radicals |
| ROS | Reactive oxygen species |
| RR | Ribonucleotide reductase |
| RVS glycoprotein | *Rhus verniciflua* Stokes glycoprotein |
| SD | Standard division |
| SEs | Synergistic Effects |
| SNL glycoprotein | *Solanum nigrum* Linne glycoprotein |
| SOD | Superoxide dismutase |
| TA | Tannic acid |
| TAS | Total antioxidant status |
| TBARS | Thiobarbituric acid reactive substance |
| TBH | Tert-butyl hydroperoxide |
| t-BHP | *Tert*-butylhydroperoxide |

| | |
|---|---|
| TBHQ | Tertiary butyl hydroquinone |
| TEAC | Trolox equivalent antioxidant capacity |
| TMBA | 3,4,5-trimethoxybenzoic acid |
| TNB | 5- thio-2-nitrobenzoic acid |
| TNF | Tumor necrosis factors |
| TO· | ∝-tocopheroxyl radical |
| TOH | ∝-tocopherol |
| TPC | Total phenolic content |
| TRAP | Total radical-trapping antioxidative potential |
| Trimidox | 3,4,5-trihydroxybenzamidoxime |
| TSC | Theoretical Scavenging Capacity |
| TSS | Total soluble solids |
| UA | uric acid |
| UA· | uric acid radical |
| UDN glycoprotein | *Ulmus davidiana* Nakai glycoprotein |
| UV | Ultra Vilot |

| | |
|---|---|
| VC | Vitamin C |
| VCEAC | Vitamin C equivalent antioxidant capacity |

# 1. INTRODUCTION

*Moringa oleifera*, *Moringa peregrina* and *Moringa stenopetala* are three species belong to a single genus family *Moringaceae* that has fourteen species, (Ganesana *et al.,* 2014).

There are limited information on the chemical, anti-nutritional contents of the flour and characterization of the oil extracted from the seeds, (Mustapha *et al.,* 2015).

*Moringa* has been regarded as a food substance since ancient times and have been used as a treatment for many diseases. The leaves, fruits, flowers and immature pods of this tree are edible and they form a part of traditional diets in many countries of the tropics and sub-tropics. Apart from its dietary importance, local folklore credits *Moringa* with a lot of herbal potency, (Ozumba *et al*, 2009).

Some of the uses of the plant include use in alley cropping, animal forage, as domestic cleaning agent, as fertilizer, for live fencing, as medicine, as ornamentals and it is resistant to most pests. Drumstick or *Moringa oleifera* is a multi-purpose tropical tree that belongs to *Moringaceae* family and has originated from Himalayan tract in Northwestern part of India, (Mendieta-Araica *et al.,* 2012 and Pandey *et al., 2011*).

Seed flour from *Moringa oleifera* is widely used as a natural coagulant for water treatment in developing countries, (Santos *et al.,* 2005).

In developing countries, *Moringa* has potential to improve nutrition, boost food security, foster rural development, and support sustainable land care, (National Research Council, 2006).

Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolic, (Anwar *et al.,* 2007).

Mature seeds yield (38–40%) edible oil called ben oil from its high concentration of behenic acid. The refined oil is clear, odorless and resists rancidity, can also be used as a natural source of behenic acid, which has been used as an oil structuring and solidifying agent in margarine, shortening, and foods containing semisolid and solid fats, eliminating the need to hydrogenate the oil. The seed cake remaining after oil extraction may be used as a fertilizer, (Rashid *et al.,* 2008).

*Moringa* flowers are used in treating malnutrition in traditional settings. The plant seeds contain hypotensive activity, strong antioxidant activity and chelating property against arsenic toxicity, (Santos *et al.,* 2009).

"Diabetes" means siphon and "mellitus" stands for sweet. Diabetes is a complex multisystem disorder characterized by a relative or absolute insufficiency of insulin secretion and disturbances in carbohydrate, protein and lipid metabolism, it is an insidious disease, (Rakesh *et al.,* 2008). Although the prevalence of diabetes is increasing, diabetes is not homogenously distributed throughout the population, (Michael, 2010).

Chronic hyperglycemia during diabetes causes gyration of body proteins that in turn leads to secondary complications affecting eyes

(retinopathy), kidneys (nephropathy), nerves (neuropathy) and arteries (atherosclerotic vascular disease). The international diabetes federation (IDF, 2014) has predicted that the number of individuals with diabetes increased from 382 million in 2014 to 592 million, in 2035 with 80% of the disease burden in low and middle-income countries, (IDF, 2014) according to recent estimation, the global population is approaching the midst of diabetes pandemic.

The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years, (Bhoomika *et al.,* 2007).

Many of traditional medicinal plants have been used successfully since ancient times to treat diabetes and related complications because plants have been the major source of drugs for the treatment of diabetes mellitus in Indian system of medicine and other ancient systems in the world, though their biologically active compounds are unknown. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes due to their effectiveness, less side effects and relatively low cost, (Rathod *et al.,* 2008).

Botanical products can improve glucose metabolism and the overall condition of individuals with diabetes not only by hypoglycemic effects but also by improving lipid metabolism, antioxidant status and capillary function, (Alam *et al.,* 2003).

Recently numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals. Based on several studies, reviews, articles and researches: Ganesana *et al.,* (2014), Mahmood, *et al.,* (2010), Adebayo, *et al.,* (2011), MPFI, (2013); the need for extensive documentation and focused research on the family as a whole and not only on some species "e.g. *Moringa oleifera"* has motivated us to bridge the information gap in this area.

Therefore, the present study aimed to comparison study by determine and evaluate (*in vitro*) the chemical composition of leaves and seeds contents of *Moringa oleifera, Moringa peregrina,* and *Moringa stenopetala* from five places under Egyptian conditions. In addition to evaluate (*in vivo*) the biological and antidiabetic effect of the extractions of *Moringa oleifera, Moringa peregrina*, and *Moringa stenopetala* leaves on STZ-induced diabetic male albino rats.

# 2. REVIEW OF LITERATURE

## 2.1 *Moringaceae* Family

There is still a great need for further scientific examination of the less utilized species in the genus, which could potentially represent a valuable commodity, both in food and medicine. Thus far, no comprehensive review has been compiled from the literature documenting the medicinal importance of all species of the family *Moringaceae*. Information in this regard is still lacking, (Chinmoy, 2007).

*Moringa* spp. are one of the most useful tropical trees with a multiple array of uses, and all plant parts of this genus are used in the indigenous systems of human medicine for the treatment of variety of ailments. *Moringa* spp. is rich sources of various phytochemical compounds including glucosinolates; however, there are only detailed profiles for *Moringa oleifera, Moringa peregrina,* and *Moringa stenopetala, (*Amaglo *et al.,* 2010).

*Moringa oleifera* commonly called the "horse-radish tree", is the most well-known and widely naturalized of the 13 species and is a native of the sub-Himalayan tracts of northwestern India. It has now become widely known as multi-purpose tree as it is grown for its nutritious pods, edible leaves and flowers and provides many beneficial properties including its use as a source of food, medicine, cosmetic oil, forage for livestock and water coagulant, (Paliwal *et al.,* 2011).

The family *Moringaceae* is also the most genetically vulnerable in their respective habitats due to a number of factors, ranging from

unsustainable exploitation and destruction by wildlife to persistent drought. Some of these species grow in rural parts of the tropics, but are not fully explored for their many properties including their medicinal and pharmacological value, and are not also fully acknowledged by the world health organization. *Moringa*-based food and medicinal products could be manufactured and exported to other parts of the world where it does not grow and can be commercialized, (Padayachee and Baijnath, 2012).

There is also tremendous potential for widespread recognition of the 13 species in the genus *Moringa* that are equally as important and valuable. Further breeding programs as well as scientific research initiatives should be undertaken for the lesser-known plant species that have great potential from a nutritional and medicinal point of view, (Padayachee and Baijnath, 2012).

*M. peregrina, M. concanensis, M. stenopetala, M. ovalifolia M. drouhardii* and *M. oleifera* are some of the less common species that have shown enormous potential for utilization in various aspects such as in nutrition, medicine, cosmetics and water coagulation. *M. rivae, M. arborea, M. borziana, M. pygmaea, M. ruspoliana, M. hildebrandtii and M. longituba* also possess great diversity as medicinal plants, (Padayachee, and Baijnath, 2012).

*Moringa oleifera* Lam. is a leguminous plant, originally from Asia, which is cultivated in Brazil and used this plant as food; there is little information about its chemical and nutritional characteristics. The characterize the leaves of *Moringa oleifera* in terms of their chemical composition, protein fractions obtained by solubility in different systems and

also to assess their nutritional quality and presence of bioactive substances, (Teixeira *et al.,* 2014).

*Moringaceae* comprises a large genus of many species in its different growth forms that are widely distributed, but less utilized. The lesser known of the 13 species in the family *Moringaceae* (Ganesana *et al.,* 2014)

*M. rivae, M. arborea, M. borziana, M. pygmaea, M. ruspoliana, M. hildebrandtii and M. longituba* also possess great diversity as medicinal plants, further research and development is critically needed for *Moringa* species and it should be focused mainly on the conservation of genetic resources, breeding programs and intensive scientific studies, (Teixeira *et al.,* 2014).

Efforts need to be expanded in this regard so that these valuable species do not go unnoticed and receive the attention they so rightfully deserve. If the genus that consists of more potentially beneficial species can be further explored and evaluated for its various properties, it can contribute to the conservation and appreciation of a natural resource, (Padayachee and Baijnath, 2012 and Teixeira *et al.,* 2014).

**2.2 Classification of Moringaceae**

The need for extensive documentation and focused research on the family as a whole and not only on *M. oleifera* has motivated us to bridge the information gap in this area, and to present a comprehensive review on the medicinal, pharmacological and phytochemical properties of the lesser known species in the family *Moringaceae; Moringa peregrina, Moringa concanensis, Moringa rivae, Moringa arborea, Moringa borziana, Moringa*

*pygmaea, Moringa ruspoliana, Moringa hildebrandtii, Moringa stenopetala, Moringa ovalifolia, Moringa drouhardii* and *Moringa longituba,* (Paliwal *et al.,* 2011).

### 2.3 Origen and distribution of *Moringa*

The countries and regions of *Moringaceae* family include Madagascar, Namibia, South West Angola, Kenya, Ethiopia, Red Sea, Horn of Africa, India, Pakistan, Bangladesh and Afghanistan in the Northwestern region of the Himalayans, (Fahey, 2005).

Almost all *Moringa* species appear to have originated in India and Africa, but have since been introduced into several countries of the tropics, (Amaglo, 2010).

*Moringa* is native to the Himalayan foothills (India/Bangladesh). As a commercial crop, it is cultivated extensively in India and parts of Africa. It would be challenging to find a region in the tropics or subtropics where *Moringa* is not grown as a backyard tree for leaf and pod consumption, medicinally and for fiber. *Moringa* is most commonly found in areas with South and Southeast Asian (particularly Filipino) populations. Today it is widely cultivated in Africa, Central and South America, Sri Lanka, India, Mexico, Malaysia, Indonesia and the Philippines. It is considered one of the world's most useful trees, as almost every part of the *Moringa* tree can be used for food or has some other beneficial property, (Paliwal *et al.,* 2011).

*Moringaceae*, a monogenetic family, with the single genus *Moringa* is characterized by (13) species of dicotyledonous tropical and sub-tropical flowering trees, (Ganesana *et al.,* 2014).

### 2.4 Botanical morphology and characteristics of *Moringa*

In developing countries, *Moringa* has potential to improve nutrition, boost food security, foster rural development, and support sustainable land care (National Research Council, 2006).

The tree is rather slender, with drooping branches that grow to approximately 10m in height. In cultivation, it is often cut back annually to (1-2) meters and allowed to regrow so the pods and leaves remain within arm's reach. The leaves, fruits, flowers and immature pods of this tree are edible and they form a part of traditional diets in many countries of the tropics and sub-tropics. The plant seeds contain hypotensive activity, strong antioxidant activity and chelating property against arsenic toxicity, (Santos *et al.,* 2009).

*Moringa* is a slender softwood tree that branches freely and can be extremely fast growing. Although it can reach three heights in excess of (10m) (33 f) and a diameter of (20-40) cm at chest height, it is generally considered a small- to medium-size tree, (Radovich, 2009).

*Moringa* Lam belongs to a single genus family *Moringaceae* grown and widely cultivated in the many countries in tropical and sub-tropical Africa (Anjorin *et al.,* 2010).

**Stem:** The stem is normally straight but occasionally is poorly formed. The tree grows with a short, straight stem that reaches a height of (1.5-2 m) before it begins branching but can reach up to 3.0 m, (Foidl *et al.,* 2001).

**Branch:** The extended branches grow in a disorganized manner and the canopy is umbrella shaped, (Paliwal *et al.,* 2011).

**Leaves:** Tripinnate compound leaves are feathery with green to dark green elliptical leaflets (1-2 cm) (0.4-0.8 in) long. The tree is often mistaken for a legume because of its leaves. The alternate, twice or thrice pinnate leaves grow mostly at the branch tips. They are (20-70) cm long, grayish-downy when young, long petiole with (8-10) pairs of pinnae each bearing two pairs of opposite, elliptic or obovate leaflets and one at the apex, (1-2 cm) long, (Morton, 1991).

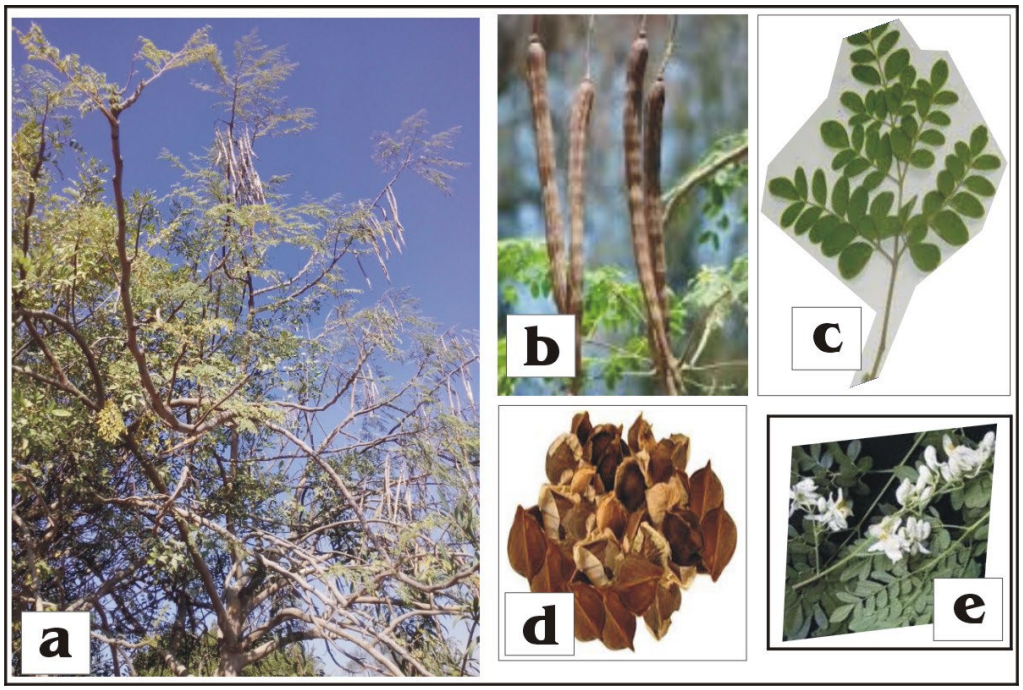


**Fig. 1 Various useful parts of *Moringa stenopetala* tree, cultivated in belbis, sharkia in Egypt, (a) tree, (b) pods (fruit),(c) leaves, (d) seeds (e) and (f) flowers.**

**Flowers:** Conspicuous, lightly fragrant flowers are borne on inflorescences 10-25 cm (4-10 in) long, and are generally white to cream colored, 2.5 cm in diameter, borne in sprays, with 5 at the top of the flower, although they can be tinged with pink in some varieties. The flowers, which are pleasantly fragrant and 2.5 cm wide are produced profusely in axillary, drooping panicles 10-25 cm long, (Sachan *et al.,* 2010).

They are white or cream colored and yellow-dotted at the base. The five-reflexed sepals are linear-lineolate. The five petals are slender-spatulas. They surround the five stamens and five staminodes and are reflexed except for the lowest, (Sachan *et al.,* 2010).

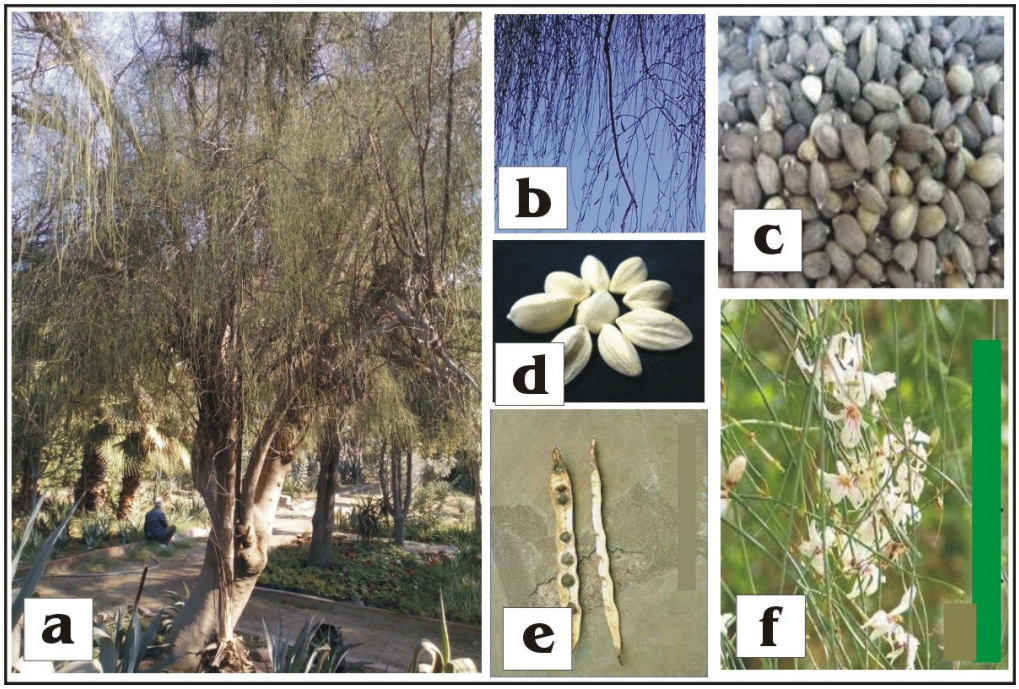


**Fig. 2. Various useful parts of *Moringa peregrina* tree, cultivated in orman botanic garden, Giza in Egypt; (a) tree, (b) leaves, (c) seeds with covers, (d) seeds without covers (e) pods (fruit) and (f) flowers.**

**Fruits:** The fruits are trilobite capsules, and are frequently referred to as pods. Immature pods are green and in some varieties have some reddish color. Pods are pendulous, brown, triangular, splitting lengthwise into three parts when dry, 30-120 cm long, 1.8 cm wide, containing about 20 sec embedded in the pith, pod tapering at both ends, 9-ribbed. Fruits production in March and April in Sri Lanka, (Sachan *et al.,* 2010).

**Seeds:** The seeds are round with a brownish semi-permeable seed hull, with three papery wings. Seed hulls are generally brown to black, but can be white if kernels are of low viability. Each tree can produce between 15,000 and 25,000 seeds/year. The average weight per seed is 0.3 g and the kernel to hull ratio is 75:25, (Ozumba *et al.*, 2009).

**Growth and development**: *Moringa oleifera* (MO) is a medium size multipurpose tree of approximately 5 to 10 m in height, which is cultivated all over the world, (Asante *et al.,* 2014).

Plants from seed can grow very rapidly under ideal conditions. Selected early flowering varieties are sometimes called annual types because they produce vegetable pods for market within a year and may be removed and new plantings established. Examples of early flowering types include 'PKM-1 and 'PKM-2' developed primarily for vegetable pod production by Tamil Nadu University in India. Early flowering types can produce market-mature pods in 6 months compared to over a year for other types. *Moringa*

varieties generally tolerate the same climatic conditions. After coppicing, branches grow quickly and immature pods are harvested in 6 months, (Rajangam *et al.,* 2001).

**Flowering and fruiting:** *Moringa* is free flowering. Flowering generally occurs 4-12 months after planting, depending on the type. Some selections flower 4-5 months after planting, (Rajangam *et al.,* 2001).

**Cultivation:** The plant is propagated by planting limb cuttings 1-2 m long, from June to August. The plant starts bearing pods 6-8 months after planting, but regular bearing commences after the second year. The tree bears for several years. It does not tolerate freeze or frost. Seed, (Rajangam *et al*., 2001), can also propagate it.

As with all plants, optimum cultivation depends on producing the right environment for the plant to thrive. *Moringa* is a sun and heat loving plant. Seeds are planted an inch below the surface and can be germinated year-round in well-draining soil. The drumstick can be grown using rainwater without expensive irrigation techniques. The yield is good even if the water supply is not. The tree can be grown even on land covered with 10-90 cm of mud, (Rajangam *et al.,* 2001).

**Scale of commercial production:** Commercial production of immature pods for processing is a large industry in India with about 1.2 million MT (metric tons) (1.1 million T) produced annually on 38,000 ha (94,000 ac), (Sharma *et al.,* 2011).

## 2.5 Environmental requirements of *Moringa*

Genus of *Moringa* is resistant to drought, because of the presence of a long tap root system. It thrives within a temperature range of 25-35 ºC, but can tolerate up to 48 ºC *Moringa* has been credited with a multitude of uses: the leaves, pods (seeds), flowers, and the growing tips of the tree are edible and nutritious, (Fuglie, 2001). Because it tolerates a wide range of soil and rainfall conditions, it is drought-resistant and is in season all year round (Ozumba, 2008). *Moringaceae*, a monogenetic family, with the single genus *Moringa,* it is native to Sub-Himalayan parts of northern India, but is now widely distributed in the tropics and sub-tropics, (Anjorin *et al.,* 2010).

**Climate:** *Moringa* is widely adapted to the tropics and subtropics. Optimum leaf and pod production requires high average daily temperatures of 25-30°C (77-86°F), well-distributed annual rainfall of 1000-2000 mm (40-80 in), high solar radiation and well-drained soils. Growth slows significantly under temperatures below 20°C (68°F). Ideal elevation is less than 600 m, above sea level (1,970 f). *Moringa* is relatively tolerant of drought and poor soils and responds well to irrigation and fertilization, (Anjorin *et al.,* 2010).

**Soils:** *Moringa* tolerates a wide range of soil types and pH (4.5-9) but prefers well-drained soils in the neutral pH range. It can grow well in heavy (clay) soils provided that they do not become saturated for prolonged periods of time. Light (sandy) soils are preferred for rooting branch cuttings directly in the ground, (Ganesana *et al.,* 2014).

## 2.6 Ecological and genetic developments

*M. oleifera* can survive in harsh climatic condition including impoverished soils without being much affected by drought (Morton, 1991). Fuglie (1999) was quick to say the "so good to be called syndrome was the biggest challenge for *Moringa*", it offers so much to us so that it's so difficult to believe if such simple plant can be so useful. The leaves of MO are considered to give immense possibilities for those who are nutritionally challenged and may be regarded as a protein and calcium supplement (Rajangam *et al.,* 2001). India is rated as the largest producer of *Moringa*, with an annual production of between 1.1 to 1.3 million tons of tender fruits from an area of 380 km² (Rajangam *et al.,* 2001). It can tolerate wide range of rainfall requirements estimated at 250 mm and maximum at over 3000 mm and pH of 5.0 to 9.0 (Palada and Chang, 2003). According to Fuglie (2000), MO plant forms the basis for several nutritional programs in many poor countries by charitable organizations, given that the leaves of MO tree are rich in essential nutrients.

*Moringa oleifera* (MO) is the most widely cultivated species of a monogenetic family, the *Moringaceae* (Fahey, 2005). Aslam *et al*. (2005) suggested the contents of different minerals in leaves and pods of M. *oleifera* to significantly differ from region to region in Pakistan. The mineral contents in *M. oleifera* and their bioavailability continue to be a subject of tremendous interest. There are however limited reports on the influence of variation in

geographical locations or agro-ecology of *M. oleifera* on the mineral composition in various organs of the plant.

Researchers at the Asian Vegetable Research and Development Center (AVRDC, 2006) reported that leaves from four different *Moringa* species (*Moringa oleifera, Moringa peregrina, Moringa stenopetala* and *Moringa drouhardii*) all contained high levels of nutrients and antioxidants.

In the past 5000 years, MO has been used as a regular component of conventional eatables in the Indian sub-continent (Anwar and Bhanger, 2003 and Anwar *et al.,* 2006).

Anjorin *et al*. (2010) confirmed that there were variations in macro and trace minerals in M. *oleifera* leaves, pods and seeds from different locations in Nigeria. The increasing conviction and confidence in the consumption of this plant calls for a need to document well the nutritional comparison of *Moringa* leaves from different agro ecological zones in Ghana. The objective of this study was to investigate and compare the nutritional components of MO leaves in two agro-ecological zones of Ghana, (Anjorin *et al*., 2010).

It has been introduced and become naturalized in other parts of India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, West Asia, the Arabian Peninsula, East and West Africa, Southern Florida, throughout the West Indies, and from Mexico to Peru, Paraguay and Brazil. In Puerto Rico, it is grown chiefly as an ornamental and in fencerows and hedges and has become naturalized along roadsides on the coastal plains and lower

foothills. The rapid growing tree was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics, (Sachan *et al.,* 2010).

*Moringa oleifera* is the best known of the thirteen species in the genus *Moringa* of family *Moringaceae*. These are *Moringa oleifera, M. arborea, M. borziana, M. concanensis, M. drouhardii, M. hildebrandtii, M. longituba, M. ovalifolia, M. peregrina, M. pygmaea, M. rivae, M. ruspoliana* and *M. stenopetala.* This fast-growing tree is grown for human food, medicine, dye, and fodder and water clarification. It has an impressive range of medicinal uses with high nutritional value. In addition to its compelling water purifying powers and high nutritional value, *M. oleifera* is very important for its medicinal value. All parts of the *Moringa* tree are edible and have long been consumed by humans, (Mahmood *et al.,* 2010).

The many uses for *Moringa* include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, bio pesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds), (Adebayo *et al.,* 2011).

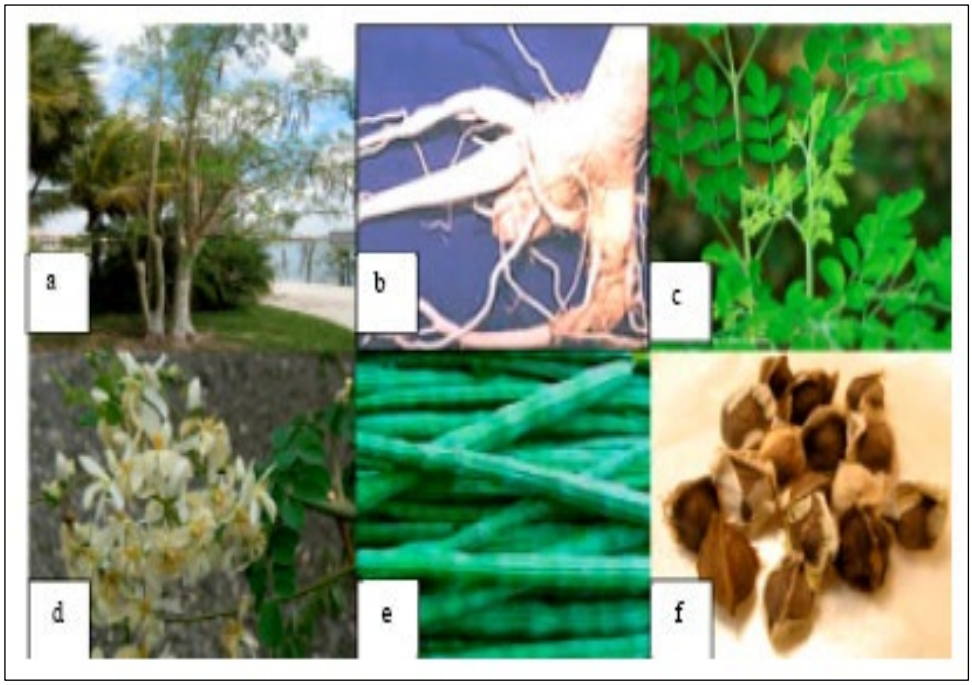



**Fig. 3. Various useful parts of *Moringa oleifera* tree, cultivated in model plant farm of nubarya in Egypt; (a) tree, (b) root, (c) leaves, (d) flowers, (e) pods (fruit) and (f) seeds.**

In the West, one of the best-known uses for *Moringa* is the use of powdered seeds to flocculate contaminants and purify drinking water. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory (Kumar *et al.,* 2009), antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia (Paliwal *et al.,* 2011).

It is generally known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil. In the light of aforementioned properties of drumstick tree the following review highlights its vernacular names, distribution, economic and commercial importance along with traditional medicine and culinary uses, (Paliwal *et al.,* 2011). While it grows best in dry sandy or loamy soil that is slightly alkaline (Anjorin *et al.,* 2010), it is adaptable to various soil conditions from 4.5 to 8.0 pH, but does not tolerate water logging, freezing or frosts conditions (Radovich, 2011).

Bamishaiye *et al*. (2011) reported that MO leaves of all stages having varying percentages of nutritional composition. The tree is an aboriginal of Indian subcontinent and has become naturalized in the tropical and subtropical areas around the world (Farooq *et al.,* 2012). Its trunk is soft, white corky and branches bearing a gummy bark. Each tripinnately compound leaves bear several small leaflets. The flowers are white and the three wing seeds are scattered by the winds (Farooq *et al.,* 2012).

Easy cultivation of *Moringa* within adverse environmental condition and wide availability attract attention for economic and health related potential in resource limited developing countries (Farooq *et al.,* 2012). In a study of Genetic diversity assessment is prerequisite for future crop improvement programed therefore, the morphological (qualitative and quantitative) and SSR makers had been used for characterization of 300 genotypes of drumstick of India; which belongs to 12 distinct populations. The study with morphological (qualitative and quantitative) and SSR makers

showed that large diversity exists in Indian germ plasm, (Ganesana *et al.,* 2014).

The population structure based grouping showed that genotypes from north India were being distributed only in two clusters out of five generated. This grouping pattern supports the idea that drumstick originated in north India and has moved to southern part and later were further diversified in southern part of India. The variation in morphological and molecular level suggests that large diversity exists in Indian genotype and diverse genotypes can be used for trait specific breeding programme particularly for oil content, which has more industrial value, (Ganesana *et al.,* 2014).

### 2.7 Nutritional values of *Moringa*

*Moringa* trees have been used to combat malnutrition, especially among infants and nursing mothers. Three non-governmental organizations in particular-Trees for Life, Church World Service and Educational Concerns for Hunger Organization (FAO, 2013) have advocated *Moringa* as natural nutrition for the tropics. *M. oleifera* leaves have essential amino acids, including the sulfur-containing amino acids in higher levels than those recommended by the food and agriculture organization (FAO, 2013), patterns similar to those of soybean seeds. Although *M. oleifera* leaves contain considerable amount of crude protein, this is mostly insoluble and has low in vitro digestibility, even after heat treatment and chemical attack, (Teixeira *et al.,* 2014).

Physical characterization of pods and seeds are given in tables (1) and (2). *Moringa* has long been considered a panacea for improving the nutrition of poor communities in the tropics and subtropics. Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Protein content of leaves is high (20-35% on a dry weight basis).

**Table 1. Physical properties of pods and seeds of *Moringa***

| **Determination** | **Area 1** | **Area 2** | **Area 3** |
|---|---|---|---|
| Average weight of pod (g) | 7.60 | - | 7.95 |
| Average weight of seeds (g)/pod | 3.59 | 5.03 | 4.83 |
| Average number of seeds (g)/pod | 12.00 | 17.00 | 16.00 |
| Average weight (g)/ 100 seeds | 29.90 | 29.60 | 30.20 |
| Average weight of kernels (g) /100 seeds | 21.20 | - | 22.50 |
| Percent weight of kernels in relation to entire seed | 72.50 | - | 74.50 |
| Percent weight of hull in relation to entire seed | 27.50 | - | 23.50 |
| Moisture in kernel (%) | 4.50 | - | 6.50 |
| Moisture in hull (%) | 9.20 | - | 12.90 |
| Moisture in whole seed (%) | 5.80 | - | 7.50 |

According to Foidl *et al*. (2001).

Most important is that the protein of high quality having significant quantities of all the essential amino acids, (Teixeira *et al.,* 2014). This amino acid balance is very unusual in plant foods. *Moringa* leaves also contain high quantities of nutrients (per 100 g fresh weight): vitamin A (7564 IU), vitamin C (51.7 mg), calcium (185 mg) and potassium (337 mg), (Foidl and Paull, 2008).

**Table 2. Analysis of *Moringa oleifera* pods, fresh (raw) has showed it to contain the following per 100 grams of edible portion**

| Factors | PODS |
|---|---|
| Moisture (%) | 86.90 |
| Calories | 26.00 |
| Protein (g) | 2.50 |
| Carbohydrate (g) | 3.70 |
| Fiber (g) | 4.80 |
| Minerals (g) | 2.00 |
| Ca (mg) | 30.00 |
| Mg (mg) | 24.00 |
| P (mg) | 110.00 |
| K (mg) | 259.00 |
| Cu (mg) | 3.10 |
| Fe (mg) | 5.30 |
| S (mg) | 137.00 |
| Oxalic acid (mg) | 10.00 |
| Vitamin A- β carotene | 0.11 |
| Vitamin B- Choline (mg) | 423.00 |
| Vitamin B1-thiamine (mg) | 0.05 |
| Vitamin B2- riboflavin (mg) | 0.07 |
| Vitamin B5- nicotinic acid | 0.20 |
| Vitamin C-ascorbic acid (mg) | 120.00 |
| Fat (g) | 0.10 |

Source: Natural nutrition for the tropics from *Moringa oleifera* by Fuglie (2001).

Analyses of the proximate composition of *M. oleifera* seeds have showed high levels of lipids and proteins with minor variations. *Moringa oleifera* is a tree distributed in Mexican semiarid and coastal regions. *M. oleifera* is used in practice in the treatment of various diseases and is available

without a medical prescription, often in the form of an herbal infusion for everyday use, (Valdez-Solana *et al.,* 2015).

Abdulkarim *et al*. (2005), have described high levels of total proteins (383.0 g $kg^{-1}$dry matter), which turned out to be greater than important leguminous seeds with respect to human nutrition, whose dry seeds usually contain 18 to 25% of protein, nearly double the contents of cereals. Moreover, as a natural source of behnic acid, the *M.oleifera* seed oil has been used as a solidifying agent in margarines and other foodstuffs containing solid and semi-solid fat, therefore eliminating hydrogenation processes, (Abdulkarim *et al*. 2005).

Pods and stem contain irrelevant amounts of tannins but saponins and alkaloids are present in amounts biologically important in leaves (80 g $kg^{-1}$), although in levels considered nontoxic to ruminants. The seed lipid content is greater than that of some soybean varieties (149-200 g $kg^{-1}$ meal), (Teixeira *et al.,* 2014).

The major saturated fatty acids present in the seeds are palmitic, stearic, arachidic and benic acids. Oleic acid is the main unsaturated fatty acid (67.9-70.0%) whose high concentration is desirable in terms of nutrition and stability during cooking and frying. In vivo studies are needed to better assess the use of this leaf as a protein source in human feed, (Teixeira *et al.,* 2014).

### 2.8 Culinary Uses

The *Moringa* pod is known as “munga”, *saragwa* or *saragwe* in India and is often referred to as drumstick in English. In South India, it is used to

prepare a variety of sambars and is also fried. In other parts of India, especially West Bengal and also in a neighboring country like Bangladesh, it is enjoyed very much. It is made into a variety of curry dishes by mixing with coconut, poppy seeds and mustard or boiled until the drumsticks are semi-soft and consumed directly without any extra processing or cooking, (Paliwal *et al.,* 2011).

It is used in curries, sambars, kormas, and dals, although it is also used to add flavor to cutlets, etc. In Maharashtra, the pods are used in sweet and sour curries called Aamatee. Tender drumstick leaves, finely chopped, are used as garnish for vegetable dishes, dals, sambars, salads, etc. It is also used in place of or along with coriander, as these leaves have high medicinal value. In some regions the flowers are gathered and cleansed to be cooked with besan to make pakoras. It is also preserved by canning and exported worldwide, (Paliwal *et al.,* 2011).

### 2.9 *Moringa* oil

From the results of comprehensive analysis of characterization of *Moringa oleifera* seed oil from drought and irrigated regions of Punjab, in Pakistan it could be concluded that drought might be considered one of the most visible factors which affected the chemical composition of some parameters of *M. oleifera* seed oils. Seed weight, oil yield, degree of unsaturation, oxidative stability and fatty acid composition are the parameters which are most vulnerable to drought. More importantly, it is evident that oil yield is the most affected by drought when compared to other parameters. As

the Punjab and Sindh provinces of Pakistan comprise vast fertile lands and irrigated plains, *M.oleifera* appears to be a potentially valuable oil seed crop to be cultivated in such irrigated regions to benefit from its potential oil. *M.oleifera* oil might be an acceptable substitute for high-oleic oils like olive and high-oleic sunflower oils as our dietary fats and it also could be used for various commodities of commercial attributes, (Anwar *et al.,* 2006).

### 2.10 Antioxidant activity and phenolic contents of *Moringa*

The DPPH• based method was first reported by (Blois, 1958) who observed the reduction of the DPPH• radical by the thiol-containing amino acid cysteine and other active compounds. Afterward (Brand-Williams *et al*. 1995) revised the original method and the DPPH• radical scavenging test became a reference point to evaluate the in vitro antioxidant capacity (Gil *et al.,* 2000).

Individual antioxidant compounds do not act alone; they act in combination with other antioxidants, as interactions among them can affect total antioxidant capacity, producing synergistic or antagonistic effects (Niki and Noguchi, 2000).

Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants have been extensively investigated. Natural antioxidants exist in nature in combination, and a combination of different antioxidants might act additively and even synergistically (Fuhrman *et al.,* 2000).

However, Fuhrman *et al*. (2000) indicated that lycopene has a strong synergistic action when combined with other dietary antioxidants in inhibiting Cu-initiated oxidation of LDL, either when the antioxidants are added to isolated LDL or when they are supplemented in the diet. Mortensen *et al*., (2001) and Cantrell and Truscott (2004) presented a synergistic effect of β-carotene with both ascorbic acid and ∝-tocopherol, with the ultimate formation of the ascorbyl radical.

Natural polyphenols exert their beneficial health effects by their antioxidant activity. These compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce ∝-tocophcrol radicals and inhibit oxidases (Alia *et al.,* 2003).

Siddhuraju and Becker (2003) studied the antioxidants properties of *Moringa* leaf extract and demonstrated that it: (1) reduced potassium ferricyanide, (2) scavenged superoxide radicals, (3) prevented the peroxidation of lipid membrane in liposomes, (4) could donate hydrogen and scavenge radicals. Aldini *et al*. (2003) and Yeum *et al*. (2003) investigated the interaction of carotenoids with other antioxidants in both the lipophilic and hydrophilic compartments of human plasma by taking advantage of both water-soluble and lipid-soluble radical initiators. Under these conditions, Yeum *et al*. (2004) demonstrated the interaction of carotenoid radicals with both ∝-tocopherol and ascorbic acid.

Overall, both methanol (80%) and ethanol (70%) were found to be the best solvents for the extraction of antioxidant compounds from *Moringa*

leaves, (Siddhuraju and Becker, 2003). Much of the total antioxidant activity of fruits and vegetables is related to their phenolic content, not only to their vitamin C content. Research suggests that many flavonoids are more potent antioxidants than vitamins C and E (Mimica-Dukic, 2005 and Oboh, 2005).

The radical-scavenging capacity was determined by the DPPH• assay which is a rapid, easy and inexpensive way. DPPH• (1,1-diphenyl-2-picrylhydrazyl) is a stable radical of organic nitrogen, characterized by a typical deep purple color and a maximum absorbance in the range of 515–520 nm. The DPPH• method is technically simple and needs only a UV–VIS spectrophotometer to perform in the presence of a hydrogen/electron donor (free radical scavenging antioxidant) the absorption intensity is decreased, and the radical solution is discolored according to the number of electrons captured (Markowicz *et al.,* 2007).

The biological activity of the natural antioxidant vitamin C can be enhanced by the presence of some other active natural antioxidants, such as gallic and tannic acid. Since many of these natural antioxidants are consumed together in foods, the potency for synergistic interactions is high in the human diet, (Ahmed *et al.,* 2011).

Natural antioxidant compounds like phenolic compounds, vitamin C, A, E, etc. exist naturally in different plant leaves, pods, seeds and fruits. These compounds possess the ability to reduce oxidative damage, that are believed to cause many diseases including cancer, cardiovascular diseases, cataracts,

atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing (Ahmed *et al.,* 2011).

## 2.11 Vitamins and elements in *Moringa*

Vitamin A was found to be at its peak during the hot-wet season, whereas iron and vitamin C was highest during the cool-dry season, (Price, 2007). Bureau of plant industry reported *M.oleifera* as an outstanding source of nutritional components. Its leaves (weight per weight) have the calcium equivalent of four times that of milk, the vitamin C content is seven times that of oranges, while its potassium is three times that of bananas, three times the iron of spinach, four times the amount of vitamin A in carrots, and two times the protein in milk (Kamal, 2008).

In addition, the leaves can serve as a rich source of beta-carotene (Nambiar and Seshadri, 2001), vitamin C and E, and polyphenolics. Also, Moringa is suggested as a viable supplement of dietary minerals. The pods and leaves of Moringa contains high amount of Ca, Mg, K, Mn, P, Zn, Na, Cu and Fe (Aslam *et al.,* 2005).

Thus, deficiency or excess amount of these microelements can alter enzyme activities and influence important biological processes in the body. As breast milk is normally the only source of food in the early stages of life, the dietary levels of the essential elements in the milk of lactating mothers are critically important. The concentrations of some essential elements determined in human milks of similar time postpartum, (Koolman and

Roehm, 2005). Iron, zinc and copper are among the micro-elements that are essential for the normal growth of infants. Iron functions as haemoglobin in the transport of oxygen and as essential component of enzymes involved in biological oxidation. Copper is necessary for the growth and formation of bone and myelin sheaths in the nervous systems. Zinc functions as a cofactor and is a constituent of many enzymes; the primary roles of zinc appear to be in cell replication and gene expression and in nucleic acid and amino acid metabolism, (Tan *et al.,* 2006).

*M.oleifera* leaf extracts are rich in pterygospermin and other related compounds such as isothiocyanates which is used in the treatment of many skin infections because of its antibiotic and fungicidal properties, (Price, 2007). Optimal growth of infants can be guaranteed only when the intake of food and water provides the required doses of all the essential elements. Calcium and magnesium are among the macro-elements that are essential for the proper growth and development of a child. Calcium, a major nutrient in human milk, contributes to the development of bones, muscle contraction, the transmission of nerve impulses and clotting of blood. Magnesium is an active component of several enzyme systems in which thymine pyrophosphate is a cofactor, (Soetan *et al.,* 2010). It is generally known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil (Bennet *et al.,* 2003).

## 2.12 Commercial uses of *Moringa*

*Moringa oleifera* is one of the most useful tropical trees. Recently it rediscovered as a multi-purpose tree with a tremendous variety of potential uses. *Moringa oleifera* is certainly under-exploited at present. It's numerous uses as a vegetable, seed oil, gum, hedge tree, ornamental and medicinal plant, and its easy propagation and cultivation justify more intensive research into its biological and economic potential. Germ plasm exist in natural stands and maintenance of long, large fruited types is usually practiced, (Paliwal *et al.,* 2011).

*Moringa* seeds have specific protein fractions for skin and hair care. Two new active components for the cosmetic industry have been extracted from oil cake. Purisoft consists of peptides of the *Moringa* seed. It protects the human skin from environmental influences and combats premature skin aging. With dual activity, antipollution and conditioning/strengthening of hair, the *Moringa oleifera* seed extract is a globally acceptable innovative solution for hair care, (Paliwal *et al.,* 2011).

## 2.13 Traditional medicine of *Moringa*

*Moringa* has been used in the traditional medicine passed down for centuries in many cultures around the word, for skin infections, anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhea, eye and ear infections, fever, glandular, swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency,

sore throat, sprain, tuberculosis, for intestinal worms, lactation ,diabetes and pregnancy, (Nikkon *et al.,* 2003). Due to hot potency it is helpful in maintaining the proper menstrual cycle. It is also helpful in relieving from skin related problems as it generates sweat in the body. The healing properties of *Moringa* oil have been documented by ancient cultures. *Moringa* oil has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner. *Moringa* oil has been used in skin preparations and ointments since Egyptian times, (Marcu, 2005). Among myriad of natural plants *Moringa oleifera* Lam. is called Miracle vegetable because of it is both a medicinal and a functional food. The plant kingdom represents a rich storehouse of traditional medicines and organic compounds that may lead to development of novel agents for various disorders. *Moringa oleifera* Lam (Syn *Moringa pterygosperma* Gaertn) commonly known by regional names such as drumstick tree, sajiwan, kelor, murungaikaai, saijhan and sajna, is a natural as well as cultivated variety of the genus *Moringa* belonging to the family *Moringaceae,* (Oduro *et al.,* 2008). Medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability, when compared with synthetic drugs, (Pracheta *et al.,* 2011). It possesses antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic and hepatoprotective activities. Considering its relevance, further research is required to explore the potential from this medicinal tree, (Ritu *et al.,* 2011). *Moringa oleifera*

possess highly therapeutic and pharmacological values, so its consumption in regular diet could possibly reduce the risk of degenerative diseases, (Paliwal *et al.,* 2011). *Moringa oleifera* is believed to possess numerous medical properties and is being used for the treatment of ascites, rheumatism venomous bites (Mishra *et al.,* 2009), enhancing cardiac function, inflammation, liver disease, cancer, hematological, hepatic and renal function. Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders, (Paliwal *et al.,* 2011). However, out of 750,000 species available on earth, only 1 to 10 % is being potentially used. *Moring*a is one such genus belonging to the family of Moringaceae, a monotypic family of single genus with around 33 species, (Arora *et al.,* 2013).

Most of these species have not been explored fully despite the enormous bioactivity reports concerning various potentials such as: cardiac and circulatory stimulants; anti-tumor; antipyretic; antiepileptic; anti-inflammatory; antiulcer; antispasmodic; diuretic antihypertensive; cholesterol lowering; antioxidant; antidiabetic; hepatic protective; antibacterial and antifungal activities, (Arora *et al.,* 2013).

## 2.14 Ethno pharmacological relevance

Over the past decade, several news-catching episodes in developed communities indicated adverse effects, sometimes life-threatening, allegedly arising as a consequence to taking herbal products or traditional medicines from various ethnic groups. Despite the popular use of *Moringa oleifera* for treating various disorders, there is limited or no scientific data available regarding safety aspects of this remedy, nor are there any documented toxicological studies that can be used to ascertain the safety index of its herbal preparation, (Awodele *et al.,* 2012).

## 2.15 Diabetes mellitus

Type II diabetes mellitus is a clinical syndrome due to relative or absolute deficiency of insulin or resistance to the action of insulin at the cellular level as a result hyperglycemia and glycosuria occurs, (Satya Narayana and Chakrapani, 2006).

Various new therapies have been introduced such as inhibition of enzymes that are involved in hydrolysis of dietary polysaccharides in the gut which can lead to reduction in blood glucose levels after a meal by reducing the absorption of monosaccharide by the entrecotes of the small intestine. Enzymes that are involved in hydrolysis of dietary polysaccharides are pancreatic amylase and glycosidase. With the advancement in treatment strategy there is risk of side effect such as excessive inhibition of pancreatic amylase may lead to abdominal distention, flatulence and diarrhea, (Hu *et al.,* 2006).

The use of herbal medicines (medicinal plants or phototherapy) has recently gained popularity in all over the world for their efficacy in Type II diabetes mellitus and some plants have minor side effects when given in large doses. But there is lack of understanding the actual mechanism of action of these medicines. These medicines are used since centuries in Unani system of medicine and they have more efficacy and fewer or no side effects therefore emphasis should be given on herbal medicine because allopathic system of medicine has failed in providing health to all. Herbal medicines are only alternative medicine that can relieve the patients. Various research studies have been carried in all over the world to evaluate the efficacy of herbs in the treatment of Type II diabetes mellitus, (Lans, 2006).

Currently available therapies for type II diabetes mellitus are as follows oral insulin secretagogues, sulfonylureas, repaglinide, nateglinide, biguanides, thiazolidinediones, alpha-glucosidase inhibitors, insulin, pramlintide and exenatide. Oral hypoglycemic drugs are valuable in the treatment of patients with type II diabetes mellitus (NIDDM). Sulphonylureas and biguanides are traditional drugs which are mainstay of treatment while there are certain new drugs available now. Insulin is used as hypoglycemic agent in Type II diabetes mellitus, (Islam and Choi, 2008).

Shanmugam *et al*., (2009) reported medicinal plants used for the treatment of type II diabetes mellitus; Type II diabetes mellitus is associated with disturbances in learning, memory, and cognitive skills in the diabetic patients. A study was conducted to evaluate the effect of chronic intraperitoneal administration of Apiumgraveolenson learning and memory

in diabetic rats using passive avoidance and Y-maze tests, (Shanmugam *et al*., 2009).

Medicinal plants have been used for the treatment of type II diabetes mellitus since ancient times, and for a long time type II diabetes mellitus has been treated orally with herbal medicines or their extracts, because plant products are frequently prescribed due to their less toxicity than conventional medicines, (Mehrdad *et al.,* 2009).

Traditional medicines are practiced worldwide for treatment of type II diabetes mellitus since ancient times. Various treatment options are available in allopathic system of medicine. The prevalence of type II diabetes mellitus is increasing in all over the world.

The need for achieving better control of blood glucose level has been evident in type II diabetes mellitus management. A wide number of herbal products are employed in the treatment of type II diabetes mellitus for their better efficacy and safety compared to synthetic medicine, (Akram, 2013).

World health organization (WHO) is also supporting the research on herbal medicine regarding type II diabetes mellitus. Various hypoglycemic agents from medicinal plants have been found that are effective and safe. The medicinal plants used for the treatment of type II diabetes mellitus were reviewed based on the ethno botanic, some chemical and biochemical reports. Various plants species belonging to different families have been documented used for treatment of type II diabetes mellitus, (Akram, 2013).

## 2.16. Streptozotocin induced diabetes mellitus

STZ is toxic to the insulin-producing beta cells of the pancreas in mammals. STZ induces sever and irreversible hyperglycemia in experimental animal, (Mitra *et al.* 1996), so it used as an animal model of diabetes. Rats with STZ-induced diabetes have reduced body weight, hyperglycemia, and hypoinslinemia because of damaged insulin-secreting cells in pancreatic islets, (Jae-Jeong *et al.,* 2003).

STZ stimulate insulin secretion from pancreatic ß cells by inhibiting ATP-sensitive potassium channels in pancreatic ß cell membrane, (Periyar *et al.,* 2009), the blood glucose level increases in diabetic rats, (Daisy *et al.,* 2009).

STZ has been widely used intravenously or intraperitoneal to induce type I diabetes in animal models, especially rats and mices, (Yin *et al.,* 2011) and it is a broad-spectrum antibiotic, glucosamine-nitrosourea compound extracted from *Streptomyces acromogenes*, (Periyar *et al.,* 2009).

# 3. MATERIALS AND METHODS

The experiments and analysis were carried out in 2014 and 2015 at the regional center for food and feed, in the fatty acids, amino acids, elements, vitamins, protein, ash, fat, energy, mass spectrum and biology laboratories.

## 3.1 Materials collection

1- Fresh *Moringa stenopetala* leaves and seeds were collected at October 2013 from Aswan Botanic Garden, Egypt.

2- Fresh *Moringa stenopetala* leaves and seeds were collected at October 2013 from Belbis, Sharkya, Egypt.

3- Fresh *Moringa oleifera* leaves and seeds were collected at February 2014 from Model Plant Farm of Nubarya, Egypt.

4- Fresh *Moringa oleifera* leaves and seeds were collected at February 2014 from Shalateen, Egypt.

5- Fresh *Moringa peregrina* leaves and seeds were collected at February 2014 from orman plant garden of Giza, Egypt.

The collected samples were purified and allowed to air and sun drying by exposing the sample in sun between times 10.00 am to 5.00 pm daily till the sample attained constant weight. Leaves and seeds were manually ground in mortar, passed through a 25 mm sieve, and then stored at 4-8 ° C in refrigerator for further analysis.

## 3.2 Chemicals

Streptozotocin (STZ), Ethanol and all chemicals were (analytical and HPLC grade) were obtained from Sigma/Aldrich chemicals company Co. (St Louis, MO, U.S.A) or Merck Ltd (Darmstadt, Germany). All standards (fatty acids >99%, GC, purity >99% by HPLC) were obtained from Sigma-Aldrich. Extraction of total lipid contents from seeds was extracted with petroleum ether 40-60 ºC(GC grade) in Soxhlet apparatus.

## 3.3 Chemical analysis

### 3.3.1 Moisture content

Moisture contents were determined by the Codex-adopted AOAC method 934.06 (1990) and Ashes by the AOAC official method 940.26 (1990).

### 3.3.2 Total lipids

Total lipids were determined according to the method described by AOAC official method (2005) using desegregation by hydrochloric acid and results were expressed as g/100 g dried matter (DM).

### 3.3.3 Total proteins

Total proteins of Moringa sp. Leaves and Seeds were determine by AOAC official method (2005).

### 3.3.4 Fiber content

Fiber content was determined by (Kurup *et al.,* 1984) method. Two grams of finely ground defatted sample were weighed and boiled with

sulfuric acid solution (0.255 mol/l) for half an hour followed by separation and washing of the insoluble residue.

The residue was then boiled with a sodium hydroxide (0.313 mol/L) solution followed by separation, washing and drying. The dried residue was weighed and ashes in a muffle furnace at 600ºC and the loss in mass were determined.

### 3.3.5 Determination of vitamin A

Vitamin A of *Moringa* sp. leaves were determined by AOAC official method (2005).

### 3.3.6 Determination of vitamin C

Vitamin C of *Moringa* sp. leaves were determined by AOAC official method (2005).

### 3.3.7 Determination of calcium, magnesium, iron, zinc and copper

Calcium, Magnesium, Iron, Zinc and Copper were analyzed using atomic absorption spectrophotometer (Sena *et al.,* 1998).

### 3.3.8 Analysis of Moringa oil

The extracted *Moringa* oil was analyzed immediately for Free Fatty Acid Percentage (FFA %), Peroxide Value (PV), Iodine Value (IV) and Saponification Value (SV) according to method of AOAC (2005).

### 3.3.9 Identification of fatty acids composition

The oil was analyzed for fatty acid composition by Gas Liquid Chromatography (GLC). After preparation of fatty acid methyl ester (Morrison and Smith 1964 and AOCS 2005) by using Boron Trifluoride (with Methanol 12%) BF3 to prepare their methylesters. Analyses were performed in Shimadzu Gas Liquid Chromatography; model GC-2010, Kyoto, Japan, equipped with auto sampler analyzer AOC-2010 and Flame Ionization Detector (FID).

Capillary column silicon based polymers (polysiloxanes), polyethylene glycols and silica solid absorbent model DB-wax, USA. Helium used as carrier gas with flow rate 30.0 ml/min.

The chromatographic conditions were as follows:

Injection sample size 1 microliter (µl). Injection port temperature 260 ºC. Column temperature was programmed to increase from 30 to 230 ºC at 5 min and then was maintained at 230 ºC for 30 min final time 58 min. Column pressure flow 0.58 ml/min; linear velocity 20.6 cm/sec and total 6.5 ml/min., Split ratio: 5/100 and Detector temperature was 260 ºC.

## 3.4 Preparation of *Moringa* leaves extract

### 3.4.1 Preparation of aqueous extract for *Moringa oleifera* leaves

To 100 g of *Moringa oleifera* leaves powdered, 500 ml portion of distilled water was added and the mixture was then boiled in a conical flask for 30 min. After the set time, the mixture was filtered, the obtained extraction

called stock solution. The aqueous extract was kept in air tight brown bottle in a refrigerator at 4 ºC until used.

### 3.4.2 Preparation of *Moringa* leaves ethanolic extract

Five hundred grams of the powdered *Moringa* leaves were soaked in 2.5 L of 70% ethanol at room temperature in a conical flask for 72 h. After the set time, the mixture was then filtered. The process was repeated 3 times and the filtrate was collected then evaporated to dryness using rotator evaporator at low temperature. A brownish residue weighing 75 gm/kg dried leaves powder (7.5%) was obtained. The extract was kept in air tight brown bottle in a refrigerator at 4 ºC until used**.**

### 3.4.3 Total phenolic contents

Total phenolic contents in the ethanolic extract were determined by slightly modified Folin-ciocalteu method. An aliquot (100 µl) of the extract of different concentration was mixed with 2.5 mL Folin-ciocalteu reagent (diluted with water 1:10 v/v) and 2 mL (75 g/l) of sodium carbonate.

The tubes were vortexed for 15 sec. and allowed to stand for 30 min at 40 °C for color development. Absorbance was recorded against reagent blank at 765 nm using UV-VIS spectrophotometer Specord 250 plus, Analytic Jena, Germany.

The total phenolic contents were expressed as mg/g gallic acid equivalent (GAE). The calculation was based on the standard curve of gallic acid, according to Ainsworth and Gillespie, (2007).

### 3.4.4 DPPH free radical-scavenging capacity assay

To measure the scavenging capacity of a single antioxidant or a mixture of antioxidants, the required amount of antioxidant was pipetted into a cuvette. These antioxidant mixtures were subjected to preincubation at room temperature for 5 min in the dark.

To start the reaction, 1 ml of DPPH in ethanol (0.3 mmol/l, stored at 0 C°) was added to 2 ml of the antioxidant mixture in a Teflon capped cuvette at room temperature. The mixtures were shaken quickly, placed into the cell holder and the absorbance was measured at 540 nm with a UV spectrophotometer (Specord 250 plus, analytic jiena, Germany).

### 3.4.5 Gas chromatography-mass spectrometry (GC-MS) analysis

Volatile compounds analysis by Gas Chromatography–Mass Spectrometry (GC–MS) analysis was carried out for the all methanolic extracts using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 μm film thickness). The carrier gas was helium with the linear velocity of 1 ml/min. The methanolic extracts were redissolved in methanol and filtered using 0.45μm pore size whatman polyethersulfone membrane filter. The analysis was performed according to the method described by Lehotay, (2002).

The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and

by computer matching with NIST and WILEY library Lehotay, (2002). as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

## 3.5 Biological experiment

### 3.5.1 Biological experiment design

A total of 72 male Albino rats weighing 170±30 gm were used for two biological experiments in this study. The first biological experiment (42 male albino rats) focused on *Moringa oleifera* leaves using three different levels of ethanolic extract (600, 450, 300 mg/kg b.w) and two different levels of aqueous extract (225 and 190 mg/kg b.w).

**Table 3. Standard recommended rodent diet composition of AIN 76**

| No. | Material | Quantity (g/1000g) |
|---|---|---|
| 1 | Sucrose | 150 |
| 2 | Casein | 200 |
| 3 | Corn Starch | 500 |
| 4 | Corn Oil | 50 |
| 5 | Cellulose | 50 |
| 6 | Mineral Mix. | 35 |
| 7 | Vitamin Mix. | 10 |
| 8 | DL-Methionine | 3 |
| 9 | Choline Bitartarate | 2 |

**Values were taken from Reeves *et al.,* (1993). AIN 76; American institute of nutrition standard reference diet for rodents.**

The second biological experiment (30 male albino rats) focused on *Moringa pregrina* and *Moringa stenopetala* leaves using the optimum extract level (450 mg/kg b.w) obtained from the first biological experiment of *Moringa oleifera.*

Animals were obtained from animal house in veterinary medicine, Cairo University, Egypt. The rats were raised in the animal's house of regional center for food and feed, agriculture research center, Giza, Egypt and kept in wire-bottomed stainless steel cages, and maintained under standard conditions (12 h light/12 h dark cycle; 20-25 ºC; R.H not less than 55%).

The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. During this period, rats were allowed free access of water and American institute of nutrition standard reference diet (AIN 76) table (3) was used as basal diets adlibitum, 1% vitamin mixture, and 3.5% mineral mixture, (Reeves *et al.,* 1993).

### 3.5.2 Induction of diabetes

Diabetes was induced by single intraperitoneal injection of streptozotocin (STZ) (65 mg/kg body weight in 0.1 mol/l citrate buffer, pH 4.5) into 16-18 h fasted rats (Katsumata *et al.,* 1992).

The STZ-treated rats were kept for the next 24 h on 5% glucose solution bottles in their cages to prevent initial drug induced hypoglycemic mortality, (Dhandapani *et al.,* 2002).

After 48-72 h of STZ injection, blood samples were collected by tail snip method and the sugar level of each animal was determined. All rats with

fasting blood glucose concentration of greater than 240 mg/dl (11.1 mmol/l) were considered hyperglycemic and selected for the experiment, (Burcelin *et al.,* 1995).

### 3.5.3 The first biological experiment

The first biological experiment was designed to screening and evaluates which concentration and extraction of MOL were more affect as natural antidiabetic in an experimental model in STZ-induced diabetic male albino rats.

A total of 42 rats were divided into 7 groups of 6 rats each as following:

Group 1 (Control) was control group rats that received basal diet of administration orally.

Group 2 (Diabetic) was diabetic group rats that received basal diet of administration orally.

Group 3 was diabetic rats that received basal diet and treated with 600 mg/kg body weight per day MOL ethanolic extract orally.

Group 4 (MOE) was diabetic rats that received basal diet and treated with 450 mg/kg body weight per day MOL ethanolic extract orally.

Group 5 was diabetic rats that received basal diet and treated with 300 mg/kg body weight per day MOL ethanolic extract orally.

Group 6 was diabetic rats that received basal diet and treated with 190 mg/kg body weight per day MOL aqueous extract orally.

Group 7 was diabetic rats that received basal diet and treated with 225 mg/kg body weight per day MOL aqueous extract orally.

### 3.5.4 The second biological experiment:

The Second biological experiment was designed to comparison and evaluates which concentration and extraction among MOL, MPL and MSL extractions were the highest affect as natural antidiabetic in an experimental model in STZ-induced diabetic male albino rats.

A total of 30 rats were divided into 5 groups of 6 rats each as following:

Group 1 (Control) was control group rats that received basal diet of administration orally.

Group 2 (Diabetic) was diabetic group rats that received basal diet of administration orally.

Group 3 (Medicine) (MED) was diabetic rats that received basal diet and treated with a single dose of Cedophage 100 mg/kg body weight per day orally.

Group 4 (MPE) was diabetic rats that received basal diet and treated with a single dose of 450 mg/kg body weight per day MPL ethanolic extract orally.

Group 5 (MSE) was diabetic rats that received basal diet and treated with a single dose of 450 mg/kg body weight per day MSL ethanolic extract orally.

At the end of the tow experiments, all male albino rats were fasted for l2h, rats were anaesthetized with $CO_2$.

The blood samples were withdrawn from retro-orbital venous plexus through capillary tube into a centrifuge tube samples were allowed to coagulate at room temperature and then serum was separated by centrifuged at 3000 rpm for 20 min.

The blood samples collected into sterilized Eppendorf tubes and stored at 0°C until analysis for AST, ALT, Creatinine, Triglycerides, Total Cholesterol, HDL and LDL.

## 3.6 Biochemical assay

### 3.6.1 Determination of serum glucose level

The blood glucose levels were measured using reagent strips with a glucometer (Accu-Check Active, Roche, Germany) in samples obtained from the tail vein. Animals with blood glucose levels of 240 mg/dl and above were accepted as diabetic rat.

### 3.6.2 Determination of aspartate amino transferase (AST) and alanine amino transferase (ALT) as liver markers.

**Principle**

The glutamic transaminase enzymes; serum glutamic oxalacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT), catalyze the transfers of the amino group of glutamic acid to oxalacetic acid and pyruvic acid in reversible reactions.

The transaminase activity is proportional to the amount of oxalic or pyruvate formed over a definite period of time and is measured by a reaction with 2,4.Dinitrophenylhydrazine (DNPH) in alkaline (Young, 2001).

**Reagent composition**

| | | |
|---|---|---|
| **R1 Buffer** | Phosphate buffer pH 7.4 | 100 mmol / l |
| | L – Aspartate | 100 mmol / l |
| | 2 – Oxoglutarate | 4 mmol / l |
| **R2 substrate** | 2 , 4 – dinitrophenyl hydrazine | 0.18 mmol / l |

**-Additional reagent: Sodium hydroxide 0.4 mol/l.**

**Preparation**

Liquid reagents must be at room temperature (+15-25°C) before using.

Ready to use liquid reagents and stable up to expiry date given on label when stored at 2-8°C.

**Assay conditions**

Wavelength: 505 nm (490-550)

Cuvette: 1 cm light path

Temperature: 37°C/ 15-25°C

**Procedure**

**Pipette test tubes**

| | **Blank** | **Specimen** |
|---|---|---|
| **Specimen** | ------- | 0.1 ml |
| **Reagent 1** | 0.5 ml | 0.5 ml |
| **Dist. Water** | 0.1 ml | ------- |

Mix, include for exactly 30 minutes at 37°C.

**Reagent 2** 0.5 ml

Mix, allow standing for exactly 20 minutes at 20 – 25°C.

**NaOH (0.4 mol/ l)** 0.5 ml

Mix, read the absorbance of specimen ($A_{specimen}$) against the reagent blank after 5 minutes. The color intensity is stable for 1 hour.

**Calculation of AST**

Obtain the activity of AST in the serum from the next table

| Absorbance | U / l | Absorbance | U / l |
|---|---|---|---|
| 0.020 | 7 | 0.100 | 36 |
| 0.030 | 10 | 0.110 | 41 |
| 0.040 | 13 | 0.120 | 47 |
| 0.050 | 16 | 0.130 | 52 |
| 0.060 | 19 | 0.140 | 59 |
| 0.070 | 23 | 0.150 | 67 |
| 0.080 | 27 | 0.160 | 76 |
| 0.090 | 31 | 0.170 | 89 |

**Calculation of ALT**

Obtain the activity of ALT in the serum as on the next table

| Absorbance | U / l | Absorbance | U / l |
|---|---|---|---|
| 0.025 | 4 | 0.275 | 48 |
| 0.050 | 8 | 0.300 | 52 |
| 0.075 | 12 | 0.325 | 57 |
| 0.100 | 17 | 0.350 | 62 |
| 0.125 | 21 | 0.375 | 67 |
| 0.150 | 25 | 0.400 | 72 |
| 0.175 | 29 | 0.425 | 77 |
| 0.200 | 34 | 0.450 | 83 |
| 0.225 | 39 | 0.475 | 88 |
| 0.250 | 43 | 0.500 | 94 |

### 3.6.3 Determination of creatinine

**Principle**

The assay is based on the reaction of creatinine with sodium picrate **(**Burtis, 1999). Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents. The intensity of the color formed is proportional to the creatinine concentration in the sample (Young, 2001).

**Reagent compositions**

| | | |
|---|---|---|
| **R 1 Creatinine Standard** | Creatinine aqueous primary standard 2 mg /dl | |
| **R 2 Picric Reagent** | Picric acid | 17.5 mmol /l |
| **R 3 Alkaline Reagent** | Sodium hydroxide | 0.29 mol/l |

**Preparation**

Working reagent (WR): Mix equal volumes of R 2 Picric reagent and R3 Alkaline reagent. The working reagent is stable for 10 days at l5-25 °C.

**Required materials**

- Spectrophotometer or colorimeter measuring at 492 nm (490-510).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

**Assay conditions**

Wavelength: 492 nm (490 –510)

Cuvette: 1 cm. light path

Temperature: 20 – 25 °C

**Procedure**

1. Adjust the instrument to zero with distilled water,
2. Pipette into a cuvette:

| | Blank | Standard | Sample |
|---|---|---|---|
| **WR (ml)** | 1.0 | 1.0 | 1.0 |
| **Standard (µl)** | --- | 100 | --- |
| **Sample (µl)** | --- | --- | 100 |

3. Mix and start stopwatch.
4. Read the absorbance (A1) after 30 seconds and after 120 seconds (A2) of the sample addition.
4. Calculate: Δ A= A2-A1.

**Calculation**

$\frac{\Delta ASample - \Delta ABlank}{\Delta AStandard - \Delta ABlank} \times 2$ (Standard con.) = mg/dl of creatinine in the sample

Conversion factor: mg /dl× 88.4 = mol/l.

## 3.6.4. Determination of serum triglycerides level

Serum triglycerides level was determined according to the enzymatic-colorimetric, End point method as described by Fossati and Prencipe (1982). Kit was provided by Greiner Diagnostic GmbH (Germany) and purchased from Indomedics Egypt Company.

**Principle:**

Enzymatic determination of Triglycerides was carried out according to the following reactions:

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-P + ADP}$$

$$\text{Glycerol-3-P + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate + } H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine + 4-Chlorophenol} \xrightarrow{\text{POD}} \text{Chinonimine + HCl} + 4H_2O$$

**Reagent Composition:**

| | |
|---|---|
| Good's Buffer, pH 7.2 | 50 mmol/l |
| 4-Chlorophenol | 4 mmol/l |
| ATP | 2 mmol/l |
| $Mg2^+$ | 15 mmol/l |
| Glycerokinase (GK) | ≥ 0.4 KU/l |
| Peroxidase (POD) | ≥ 2.0KU/l |
| Lipoprotein lipase (LPL) | ≥ 4.0 KU/l |
| 4-Aminoantipyrine | 0.5 mmol/l |
| Glycerol-3-phosphateoxidase (GPO) | ≥ 1.5 KU/l |
| **Standard** | 200mg/dl |

**Procedure:**

| | |
|---|---|
| Wavelength | 500 nm (492-550) |
| Temperature | 37°C |
| Cuvette | 1 cm light path |

1- One ml of Reagent (1) was pipetted in three test tubes, labeled as follows blank, standard and sample.

2- 10 µl of the Standard was added to the standard test tube and 10 µl of the serum sample was added to the sample test tube.

3- Solution in the three tubes was mixed, and the optical density (OD) was read after 5 minutes incubation.

**Calculation:**

OD sample / OD standard X C

Where C=200 in case of concentration was measured in mg/dl

C= 2 in case of concentration was measured in g/l

C=2.26 in case of concentration was measured in mmol/l

C= standard concentration

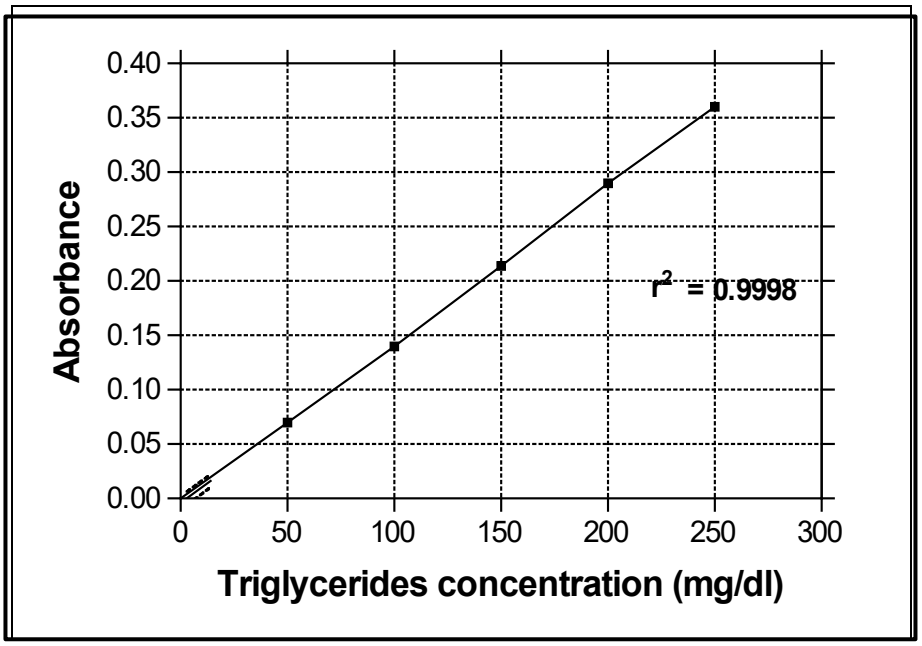



**Fig. 4 Standard calibration curve of triglycerides**

### 3.6.5. Determination of serum total cholesterol level:

Serum total cholesterol level was determined according to the enzymatic- colorimetric end point method as described by Allain (1974). The kit was provided by Greiner Diagnostic GmbH (Germany), and purchased from (Indomedics Egypt Company).

**Principle:**

Cholesterol Esterase enzyme breaks down cholesterol esters into cholesterol and fatty acids, the produced cholesterol reacts with oxygen and is converted into cholestan-3-one and hydrogen peroxide. Hydrogen peroxide reacts with phenol and 4-aminoantipyrine producing Red quinone (Colored complex). The color intensity of red quinone was measured at 500 nm (492-550).

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Chol. Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestan-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinone} + 4\ H_2O$$

**Reagents:**

**R1:**

| | |
|---|---|
| Phosphate buffer (*pH* 6.5) | 30.0 mmol/l |

| | |
|---|---|
| 4-Aminoantipyrine | 0.25 mmol/l |
| Phenol | 25.0 mmol/l |
| Peroxidase (POD) | >5.0KU/l |
| Cholesterol esterase | 150.0 U/l |
| Cholesterol oxidase (CHOD) | >100.0 U/l |
| **Standard** | 200 mg/dl |

**Procedure:**

| | |
|---|---|
| Wavelength | 500 nm (492-550) |
| Temperature | 37°C |
| Cuvette | 1 cm light path |

1- One ml of Reagent (1) was pipetted in three test tubes, labeled as follows Blank, Standard and Sample.

2- 10 µl of the Standard was added to the Standard test tube and 10 µl of the serum sample was added to the Sample test tube.

3- Solution in the three tubes was mixed, and the optical density (OD) was read after 5 minutes incubation.

**Calculation:**

OD sample / OD serum X n

Where n=200 in case of concentration was measured in mg/dl

n= 2 in case of concentration was measured in g/l

n=5.17 in case of concentration was measured in mmol/l

n= standard concentration

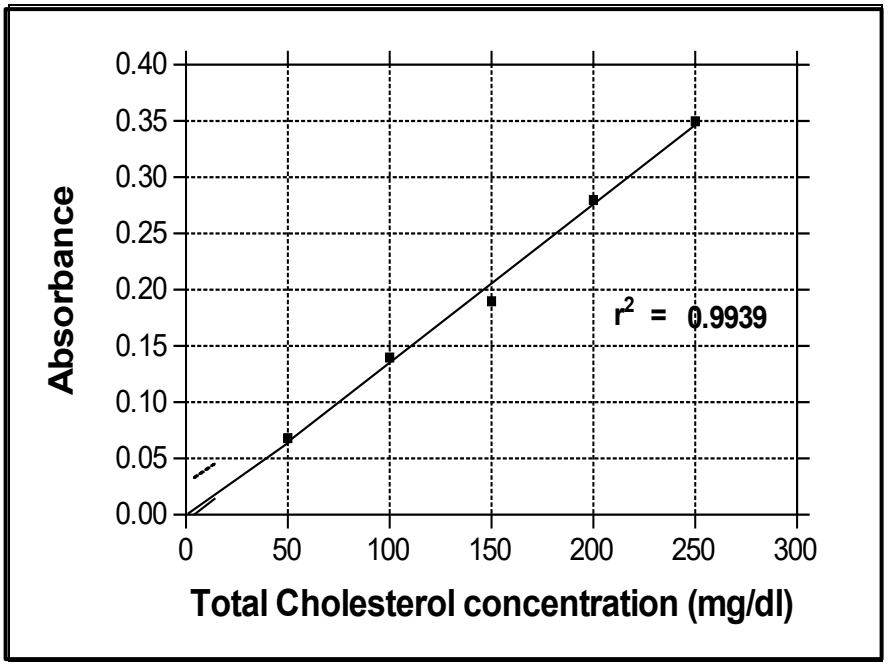



**Fig. 5** Standard calibration curve of total cholesterol

### 3.6.6. Determination of serum HDL- cholesterol level:

Serum HDL-Cholesterol level was determined according to the precipitation, phosphotungestic acid method as described by Burstein *et al*. (1970). Kit was provided by Greiner Diagnostic GmbH (Germany) and purchased from Indomedics Egypt Company.

**Principle:**

Chylomicrons, Very Low Density Lipoproteins (VLDL) and Low Density Lipoproteins (LDL) of serum are precipitated by Phosphotungestic acid and Magnesium ions.

After centrifugation, High Density Lipoproteins (HDL) is in the supernatant. Cholesterol included in this phase was measured by an enzymatic method.

**Reagents:**

**R1:**

| | |
|---|---|
| Phosphotungestic acid | 0.55 mmol/l |
| Magnesium chloride | 25 mol/l |

**Additional reagent:** Kit for cholesterol determination.

**Procedure:**

1-Sample preparation:

100 µl of serum sample was added to 200 µl of precipitating reagent. The solution was mixed well, incubated for 10 minutes and was centrifuged at 4000 r.p.m. for 10 minutes.

2-HDL determination:

The cholesterol kit was used for HDL-Cholesterol determination as followed:

| | |
|---|---|
| Wavelength | 500 nm (492-550) |
| Temperature | 37°C |
| Cuvette | 1 cm light path |

1- One ml of Reagent (1) was pipetted in three test tubes, labeled as follows Blank, Standard and Sample.

2- 100 µl of the Standard was added to the Standard test tube, 100µl distilled water was added to the Blank test tube and 100 µl of the serum sample was added to the Sample test tube.

3- Solution in the three tubes was mixed, and the optical density (OD) was read after 5 minutes incubation.

**Calculation:**

OD sample / OD standard X n

Where n=200 in case of concentration was measured in mg/dl

n= 2 in case of concentration was measured in g/l

n=standard concentration

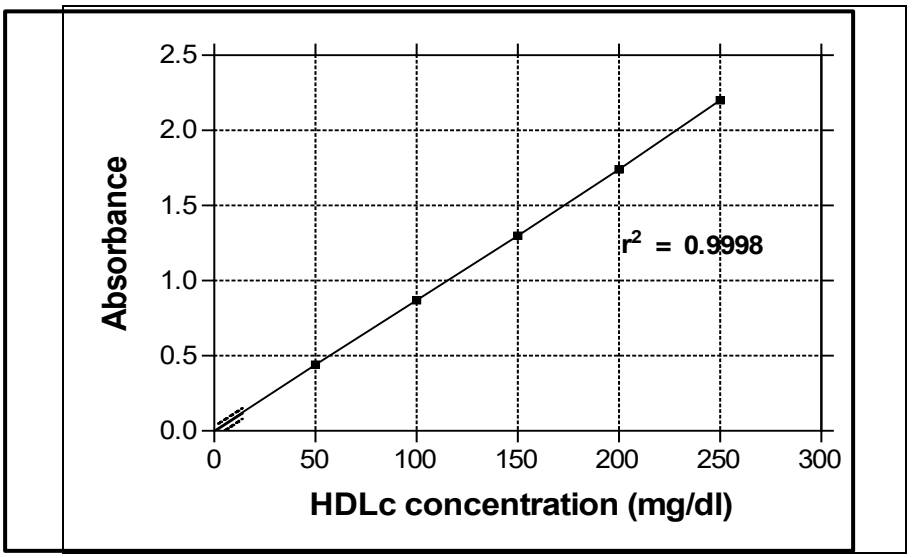



**Fig. 6. Standard calibration curve of HDL-C**

### 3.6.7. Determination of serum LDL- cholesterol level:

Serum LDL-Cholesterol level was determined according to the precipitation, Heparin/Citrate method as described by Martin *et al*. (1986). Kit was provided by Greiner Diagnostic GmbH (Germany) and purchased from Indomedics Egypt Company.

**Principle:**

Low Density Lipoproteins (LDL) from the serum sample are precipitated by heparin/ sodium citrate.

After centrifugation High Density Lipoproteins (HDL) and Very Low Density Lipoproteins (VLDL) stay in the supernatant. They were measured

by common enzymatic cholesterol determination. This value was subtracted from the Total Cholesterol and yielded the final result of LDL-Cholesterol.

**Reagents:**

**Precipitation reagent composition**

| | |
|---|---|
| Heparin | 0.68 g/l (corresponding to 100 IU /l) |
| Sodium citrate | 0.064 mol/l |
| Stabilizers | |

**Additional reagent:** Kit for cholesterol determination.

**Procedure:**

Precipitation:

1- Into Centrifuge tubes 100µl of the serum sample was mixed with 1000µl precipitation reagent and was incubated at room temperature.

2- Centrifuge tubes was centrifuged for 10 minutes at >4000 r.p.m.

3- The clear supernatant was used for determination in between 2 hours.

Determination of Cholesterol $_{supernatant}$:

| | |
|---|---|
| Wavelength | 500 nm |
| Temperature | 20-37°C |
| Cuvette | 1 cm light path |

Into 2 Cuvettes labeled as followed Sample cuvette and Blank cuvette 100 µl of sample supernatant was mixed with 1000µl of cholesterol reagent and was incubated for 5 minutes then was read against reagent blank.

**Calculation:**

Cholesterol $_{supernatant}$ (mg/dl) = reading X $F_1$

Cholesterol $_{supernatant}$ (mmol/l) = reading X $F_2$

| Factors | mg/dl | mmol/l |
|---|---|---|
| *Wavelength* | $F_1$ | $F_2$ |
| *500 nm* | *660* | *17.1* |

LDL-Cholesterol = Cholesterol $_{Total}$ – Cholesterol $_{Supernatant}$

## 3.7. Histological technique preparation of liver, kidney and pancreas tissues examination

Animals were killed by cervical decapitation and their lung, spleen, heart, liver, kidney and pancreas tissues were rapidly removed, washed with ice-cold, 0.9% NaCl (w/v) to remove the blood, those livers were weighed by the digital top balance.

The liver, kidney and pancreatic tissues were dissected out of all the experimental animals and washed on ice cold saline immediately. A portion of the liver, kidney and pancreatic tissue was fixed in 10% formalin fixative solution for histological studies.

After fixation, tissues were embedded in paraffin, solid sections were cut at 5 μm and the sections were stained with haematoxylin and eosin. The stained section on the slide was covered with a thin glass to protect the tissue from being scratched in order to provide better optical quality for viewing under microscope at a magnification of ×250, (Strate *et al.,* 2005).

### 3.8. Statistical analysis

The analysis was carried out in triplicates for all determinations and the results of the triplicate were expressed as mean±SE. The SPSS program (version 15.0 SPSS Inc., Chicago, IL, USA) was used for the analysis of variance followed by the new Duncan's multiple range test for multiple comparisons of the means, (Duncan, 1955) $P<0.05$ between mean values were considered statistically significant.

# 4. RESULTS AND DISCUSSION

## 4.1 Moisture and ash analysis for leaves and seeds of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

The data presented in table (4) illustrated by figures (7 and 8), revealed that moisture percentages of leaves recorded high significant values more than the seeds values, recorded range (8.1-10.5%) for leaves and (5.1-7.6%) for seeds, may due to many factors; environments, climate, origin, genetic, fertilization and temperature range. MON leaves recorded the highest significant value (10.5%), followed by MSB, MOS and MPG (9.5, 9.5 and 9.4 %) respectively.

**Table 4. Determinations of moisture and ash percentages of leaves and seeds for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala***

| Moringa sp. | Leaves | | Seeds | |
|---|---|---|---|---|
| | Moisture% | Ash% | Moisture% | Ash% |
| MSB | $9.50^{a}\pm0.04$ | $9.50^{b}\pm0.10$ | $5.47^{c}\pm0.04$ | $3.70^{a}\pm0.12$ |
| MSA | $8.06^{b}\pm0.05$ | $11.23^{a}\pm0.05$ | $5.70^{c}\pm0.05$ | $4.08^{a}\pm0.14$ |
| MON | $10.52^{a}\pm0.05$ | $9.09^{b}\pm0.04$ | $7.60^{a}\pm0.06$ | $3.23^{b}\pm0.11$ |
| MOS | $9.51^{a}\pm0.05$ | $10.43^{a}\pm0.06$ | $7.03^{b}\pm0.06$ | $3.29^{b}\pm0.13$ |
| MPG | $9.36^{a}\pm0.13$ | $10.82^{a}\pm0.04$ | $5.10^{d}\pm0.05$ | $2.87^{c}\pm0.10$ |
| LSD | 0.22 | 0.19 | 0.19 | 0.42 |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

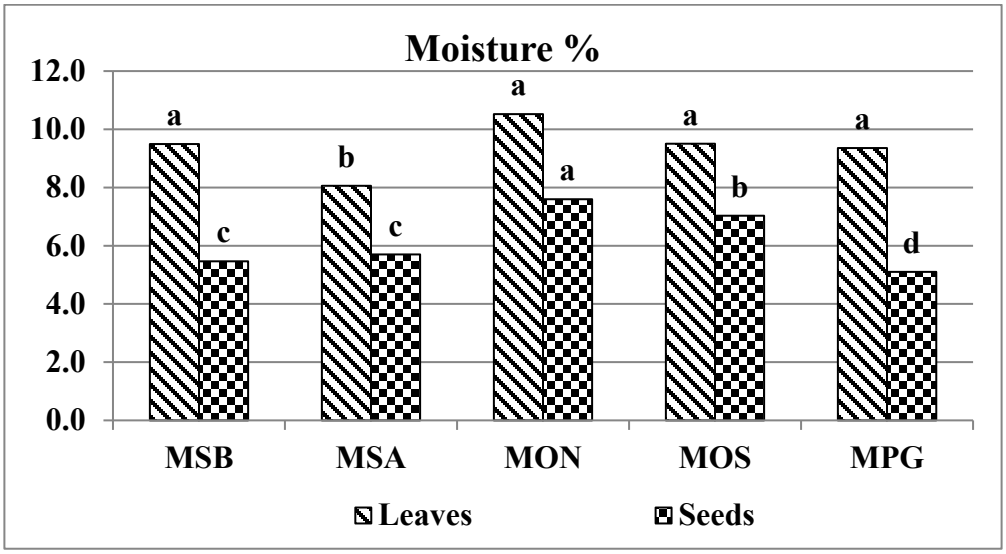



**Figure 7. Moisture percentage of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

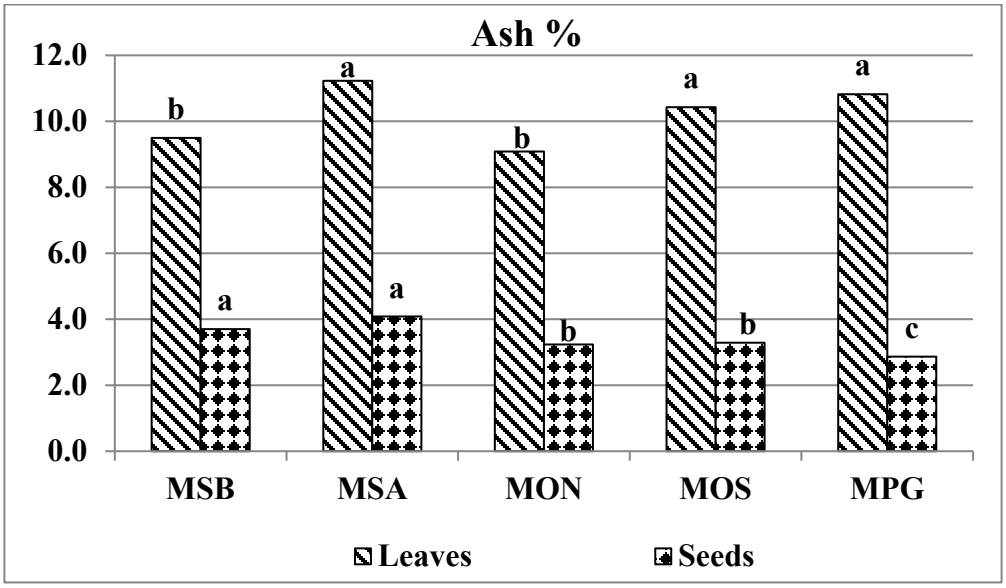



**Figure 8. Ash percentage of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

On the other hand MSA leaves recorded the lowest significant value of moisture (8.1%), in agreement with moisture value of *Moringa oleifera* leaf (9.00 ± 2.30%) reported by Sodamade *et al.,* (2013).

Moisture value is very important in food and determines the rate of food absorption and assimilation within the body, it also determines the keeping quality of food. The reported value indicated that *Moringa oleifera* leaves may not be stored at room temperature for a long period of time, these may due to many factors; environments, climate, origin, genetic, fertilization and temperature range.

Ash values of *Moringa* leaves recorded high values more than the seeds, range (9.1-11.2%) for leaves and (2.87-4.08%) for seeds.

MSA leaves recorded the highest significant value (11.2 %), followed by MPG and MOS recorded (10.8 and 10.4 %) respectively, while MSB and MON leaves recorded the lowest significant values (9.5 and 9.1 %) respectively and these values were higher than (6.00 ± 0.63%) ash content of *Moringa oleifera* reported by Sodamade *et al.,* (2013).

On the other hand MSA seeds recorded the highest significant value of ash (4.08%) followed by similar result of MSB recorded (3.7%), while MOS and MON recorded similar result with no significant difference (3.29 and 3.23%) respectively. On the other hand MPG seeds recorded the lowest value of ash (2.87%), these different may be due to many factors; environments, climate, origin, genetic, fertilization and temperature range. Ash value is very important on food determine largely the extent of mineral

matters likely to be found on food substance, the reported value of ash indicated that they were good source of mineral element.

Despite the difference in high temperatures in the south from the north in Egypt , but there is no significant difference between one type of *Moringa* plant , when analyzing the results of the moisture percentage in the *Moringa* leaves for MOS in Shalateen and MON in Nubarya recorded (9.5 and 10.5%) respectively. The differences between the two different types MSA and MPG for moisture percentage due to vegetation type, recorded (8.1 and 9.5%) respectively, not the climate.

There are significant differences in one kind of plant M. *oleifera* in Nubarya MON and Shalateen MOS has the moral of this difference is due to the geographical location or the home of agriculture, it has clearly emerged in the results of ash percentage for MON and MOS recorded (9.1 and 10.4%) respectively.

## 4.2 Protein and fat analysis of leaves and seeds for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

The data presented in table (5) illustrated by figures (9 and 10), revealed that seeds of *Moringa* like other legumes are a good source of fat and protein. In this study, the total fat yield range found to be (25.5 to 32.2%) and the data revealed also that the oil were yellow in color and had acceptable odor.

MSB seeds recorded the heights value (32.2%) followed by MSA in similar result (31.2%) followed by MOS, MPG and MON seeds recorded

(27.7, 25.7 and 25.5%) respectively, these special interest results in agreement with previous studies in Pakistan, Anwar *et al.* 2006, found that *Moringa* seed fat value range (29.6-31.3%).

Also, Mehanni *et al.,* (2014 ) and Abiodun *et al.,* (2012), found that *Moringa* seed fat value (33.3%). On the other hand, the fat value of *Moring*a *peregrina* seeds from Saudi Arabia recorded (44.90%) (Manzoor, *et al.,* 2007), these different may be due to many factors; environments, climate, origin, genetic, fertilization and temperature range.

**Table 5. Determinations of protein and fat percentages of leaves and seeds for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala***

| Moringa sp. | Leaves | | Seeds | |
|---|---|---|---|---|
| | **Protein%** | **Fat%** | **Protein%** | **Fat%** |
| **MSB** | $16.51^{c}\pm0.06$ | $5.3^{a}\pm0.03$ | $24.8^{c}\pm0.12$ | $32.2^{a}\pm0.14$ |
| **MSA** | $18.81^{b}\pm0.04$ | $2.4^{c}\pm0.27$ | $23.5^{d}\pm0.17$ | $31.2^{a}\pm0.12$ |
| **MON** | $28.28^{a}\pm0.10$ | $3.5^{b}\pm0.04$ | $29.9^{a}\pm0.15$ | $25.5^{c}\pm0.14$ |
| **MOS** | $21.60^{b}\pm0.05$ | $3.6^{b}\pm0.04$ | $28.1^{b}\pm0.03$ | $27.7^{b}\pm0.12$ |
| **MPG** | $12.27^{d}\pm0.09$ | $6.2^{a}\pm0.02$ | $30.0^{a}\pm0.07$ | $25.7^{c}\pm0.09$ |
| **LSD** | **0.22** | **0.40** | **0.38** | **0.38** |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

*Moringa* seeds have a high nutritional value; contain large amounts of protein (23.5 to 30.0g/100 g dry weight). MPG recorded the highest significant value (30%) followed by MON in similar result recorded (29.9%), followed by MOS, MSB and MSA recorded (28.1, 24.8 and 23.5%) respectively, these results were in agreement of (Abiodun *et al.,* 2012) and (Abdel-Baky and El-Baroty 2013).

The result revealed that *Moringa oleifera* MON leaves contained appreciable amount of crude protein (28.3 ± 0.16) this value is higher than (17.01 ± 0.10) reported for *Moringa* leaf by (Ogbe and John 2012) and (27.51 ± 0.00) reported by (Oduro *et al.,* 2008).

*Moringa* leaves have a high nutritional value; contain large amounts of protein (12.3-28.3 % dry weight), MON record the highest value (28.3±0.1%) followed by MOS, MSA and MSB (21.6±0.1, 18.8 and 16.5%) respectively, in agreement with the whole leaf flour contained (28.7%) crude protein, (Teixeira *et al.,* 2014).

On the other hand MPG recorded the lowest significant decrease (12.3%), these different may be due to many factors; environments, climate, origin, genetic, fertilization and temperature range.

*Moringa* leaves contain small amounts of fat (2.4-6.2% dry weight), MPG leaves recorded the highest significant value (6.2%) followed by MSB (5.3%) followed by MOS and MON leaves recorded a similar result (3.6 and 3.5%), respectively.

MSA leaves recorded the lowest value (2.4%), in agreement with the result recorded by Teixeira *et al.,* (2014), found that the obtained fat value of *Moringa* leaves recorded (7.1%).

The recorded result of crude fat value of *Moringa* leaves were (2.4-6.2%) higher than (2.43±0.47%) reported by (Sodamade *et al.,* 2013) and higher than 0.5% reported for *Moringa oleifera* powder (Mensah *et al*., 2012) and higher than (2.73±0.03%) reported for *Moringa oleifera* (Oduro *et al.*, 2008).

Fat in food determine the amount of energy available, a diet providing 1-2% of its caloric energy as fat is said to be a sufficient to human beings as excess fat consumption yields certain cardio vascular disorder such as atherosclerosis, cancer and aging (Davidson et al., 1975) and (Corel et al., 2002).

On the other hand, the recorded result of crude fat value of *Moringa* leaves were (2.4-6.2%) is suitable enough to avoid leaves damage and break down, these different may be due to many factors; environments, climate, origin, genetic, fertilization and temperature range.

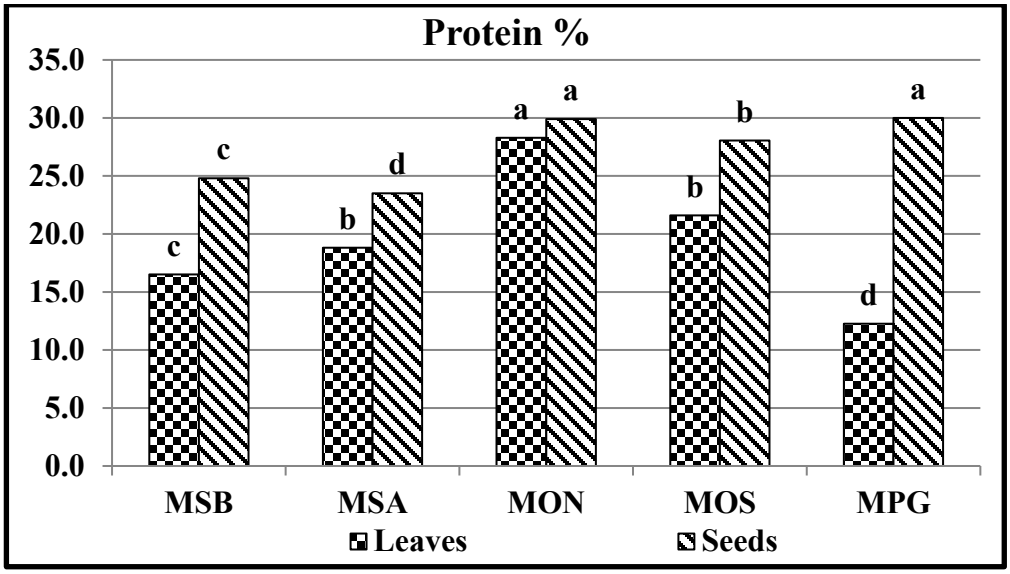



**Figure 9. Protein percentage of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

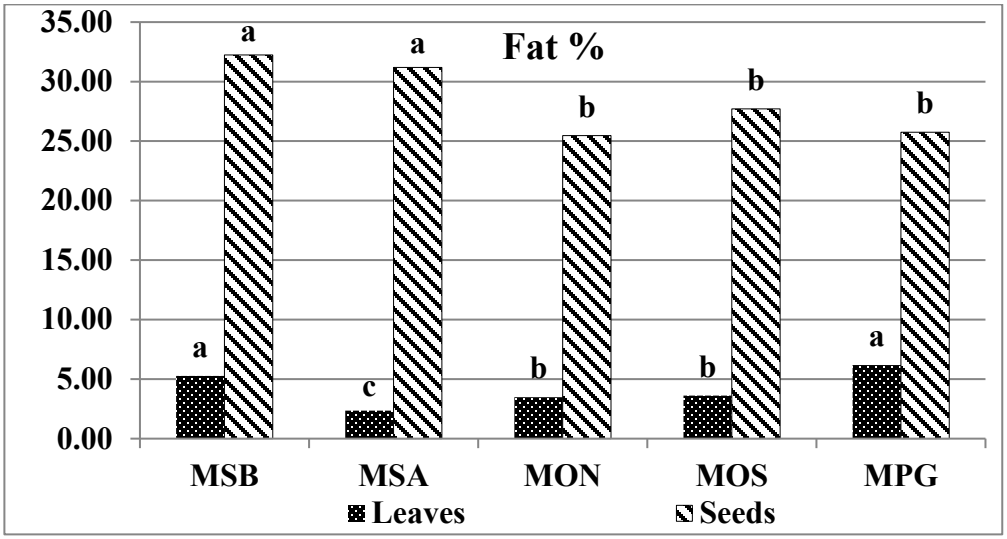



**Figure 10. Fat percentage of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

## 4.3. Fiber and NFE analysis of leaves and seeds for *Moringa oleifera, Moringa peregrina and Moringa stenopetala.*

Referred to the tabulated data in table (6), illustrated by figure (11) *Moringa* leaves contain high significant amount of fiber (21.8-30.7%) dry matter, more than *Moringa* seeds range (8.8-16.5%) dry matter.

MSB leaves recorded the highest significant value of fiber (30.7% dry matter), followed by MSA recorded a similar result (30.5%) followed by MOS recorded (24.9%) followed by MPG and MON recorded a similar result (22.2 and 21.8%), respectively, there is not agreement with (Nambiar, 2006), which showed *Moringa* leaves contain small amount of fiber 0.9 g/100 g fiber.

The value of crude fiber obtained for *Moringa* leaves were (22.2-30.7) higher than (9.25 ± 0.007) reported for *Moringa* leaf (Oduro *et al.,* 2008), and higher than (3.5% ) reported for *Moringa* leaf meal (Elkhalifa, 2007). Fiber taken as part of diet cleanses the digestive tract by removing potential carcinogens from the body and hence prevents the absorption of excess cholesterol. Fiber also adds bulk to food and reduces the intake of excess starchy food, which is the characteristics of the diet of the indigenes in this locality and hence guards against metabolic conditions such as hypertension and diabetics mellitus.

**Table 6. Determinations of fiber and NFE percentages of leaves and seeds for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala***

| *Moringa sp.* | Leaves | | Seeds | |
|---|---|---|---|---|
| | Fiber % | NFE* % | Fiber % | NFE* % |
| **MSB** | $30.7^{a}\pm0.06$ | $28.50^{bc}\pm0.25$ | $13.4^{c}\pm0.04$ | $20.42^{b}\pm0.14$ |
| **MSA** | $30.5^{a}\pm0.13$ | $29.07^{b}\pm0.13$ | $13.1^{c}\pm0.05$ | $22.39^{ab}\pm0.17$ |
| **MON** | $21.8^{c}\pm0.12$ | $26.83^{c}\pm0.03$ | $8.8^{d}\pm0.04$ | $25.02^{a}\pm0.15$ |
| **MOS** | $24.9^{b}\pm0.12$ | $29.93^{b}\pm0.17$ | $14.5^{b}\pm0.04$ | $19.47^{b}\pm0.12$ |
| **MPG** | $22.2^{c}\pm0.06$ | $39.17^{a}\pm0.29$ | $16.5^{a}\pm0.06$ | $19.75^{b}\pm0.13$ |
| **$LSD_{(0.05)}$** | **0.32** | **0.45** | **0.15** | **0.38** |

**(*) NFE were calculated by difference. Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

Referred to the tabulated data in table (6), illustrated by figure (12) *Moringa* leaves contain high amount of Nitrogen-Free Extract (NFE) (26.83-39.17%) dry matter, more than *Moringa* seeds (19.47-25.02%) dry matter. MPG leaves have the highest value of NFE recorded (39.17%) dry matter, followed by MOS, MSA and MSB in a similar result recorded (29.93, 29.07 and 28.5%), respectively, while MON leaves recorded the lowest value (26.83%).

*Moringa* seeds also contain considerable amount of fiber (8.8-16.5%), MPG seeds have the highest value of fiber, recorded (16.5%) dry matter , followed by MOS (14.5%), followed by MSB and MSA in a similar result recorded (13.4 and 13.1% dry matter) respectively, while MON seeds have the lowest value of fiber recorded (8.8%) dry matter.

*Moringa* seeds also contain high amounts of NFE (19.45-25.02% dry matter). MON seeds have the highest value of NFE, recorded (25.02%) dry matter , followed by MSA in a similar result recorded (22.39%), followed by MSB, MPG and MOS in a similar result recorded (20.42, 19.75 and 19.47% dry matter) respectively.

These special results were higher than the previous studies of *Moringa* seeds NFE (17.23%), (Mehanni *et al.,* 2014) and higher than carbohydrate or NFE content of *Moringa oleifera* vegetable leaf protein concentrates recorded (3.82 ± 0.31) by (Sodamade et al., 2013), but yet the proportion observed for *Moringa* leaf is adequate enough to meet the required energy.

Carbohydrates or NFE and lipid are the principal sources of energy. The values of carbohydrates content in these samples per 100g can provide a lower calorie of energy.

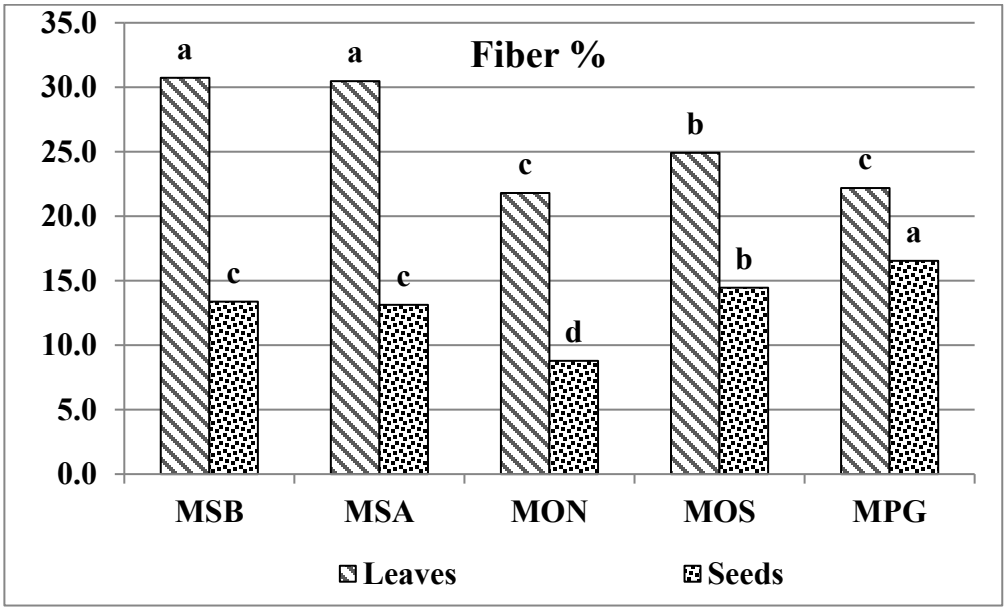



**Figure 11. Fiber percentage of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

On the other hand, these special results were lower than *Moringa* seeds values of NFE from another previous study recorded (54.61 to 57.61 %.) (Valdez-Solana *et al.,* 2015) and NFE recorded (44.4%), (Teixeira *et al.,* 2014). Due to these leaves and seeds are a potentially good source of nutritional values and different may referred to many factors; environments, climate, origin, genetic, fertilization and temperature range.

## 4.4. Energy of seeds and leaves for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

Data tabulated in table 7 and figure 13 indicated that *Moringa* has high nutritional value; seeds and leaves contain large amounts of energy (3839 to 6623 kcal/kg) dry matter.

MPG seeds recorded the highest value (6623 kcal/kg dry matter), followed by MON recorded (5840 kcal/kg dry matter), followed by MSA and MSB in a similar result recorded (5312 and 5287 kcal/kg dry matter) respectively, while MOS recorded the lowest value (4843 kcal/kg dry matter).

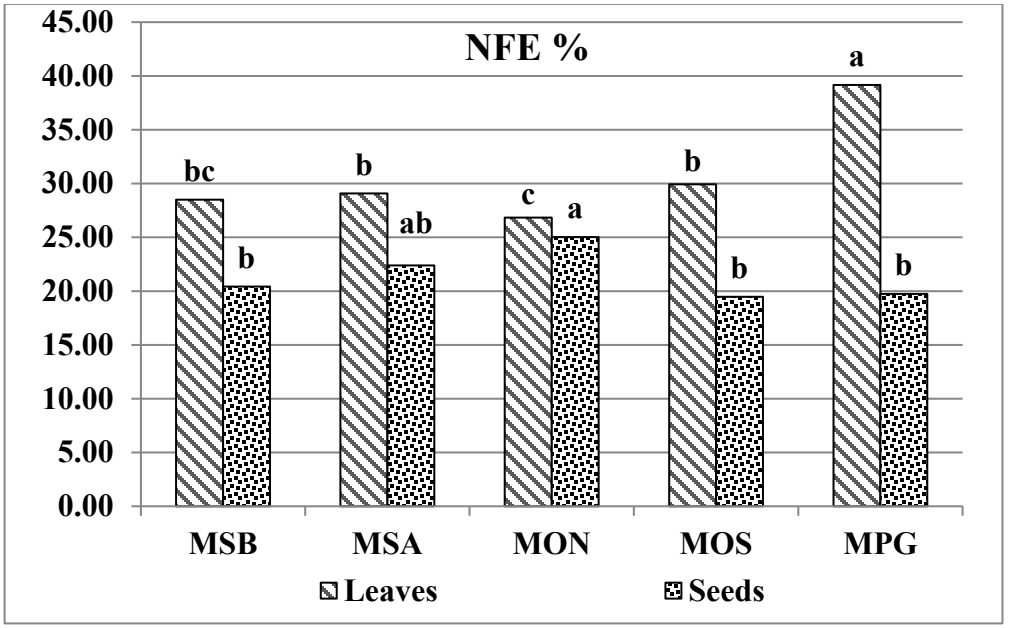



**Figure 12. NFE percentage of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

MSB leaves recorded the highest value (4214 kcal/kg dry matter), followed by MON recorded (4179 kcal/kg dry matter), followed by MOS and MSA in a similar result recorded (3965 and 3956 kcal/kg dry matter) respectively, while MPG recorded the lowest value (3839 kcal/kg) dry matter.

These interest result in agreement with previous studies reported different chemical contents of *Moringa* leaves, calculated gross energy of *Moringa* leaves was 4468 kcal/kg, (El-Badawi *et al.,* 2014).

**Table 7. Energy values of seeds and leaves for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala***

| *Moringa Sp.* | Leaves Energy (kcal/kg) | | | Seed Energy (kcal/kg) | | |
|---|---|---|---|---|---|---|
| **MSB** | 4214[a] | ± | 5.93 | 5287[c] | ± | 2.96 |
| **MSA** | 3956[c] | ± | 3.18 | 5312[c] | ± | 5.81 |
| **MON** | 4179[b] | ± | 4.36 | 5840[b] | ± | 4.41 |
| **MOS** | 3965[c] | ± | 2.89 | 4843[d] | ± | 4.93 |
| **MPG** | 3839[d] | ± | 4.36 | 6623[a] | ± | 2.33 |
| **LSD (0.05)** | **22.4** | | | **31.7** | | |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

There were balance in the results of energy values for leaves and seeds as shown in table (7) and figure (14); the value of energy for seeds is higher than the value of energy for leaves. These different may referred to many

factors; environments, climate, origin, genetic, fertilization and temperature range.

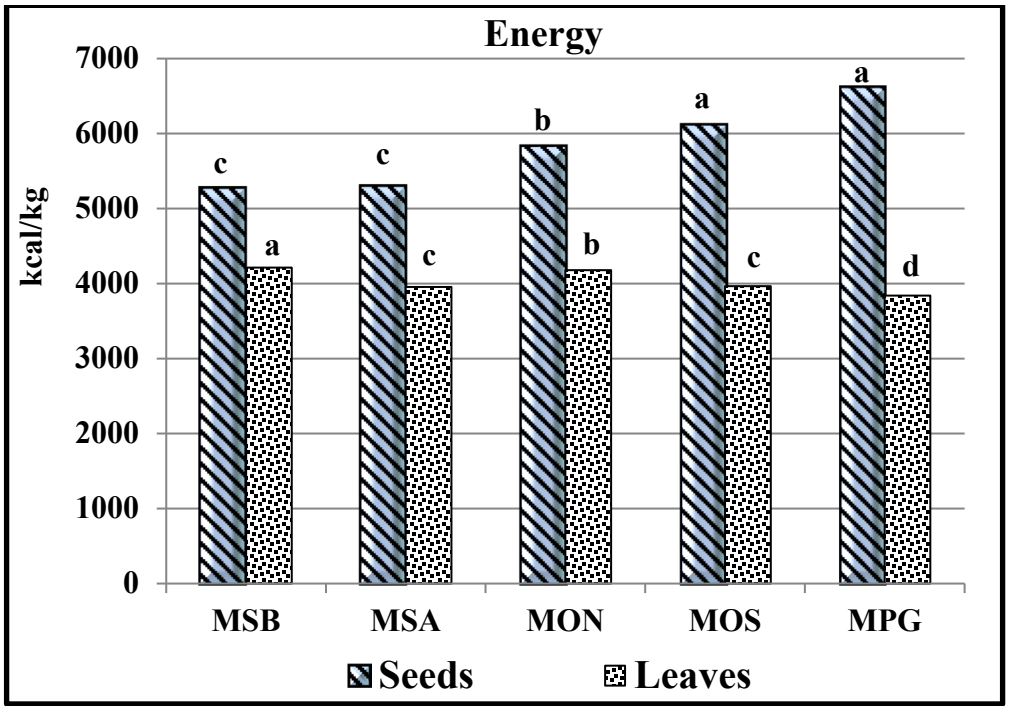



**Figure 13. Energy of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

## 4.5. The elements analysis of leaves for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

The data presented in table (8) showed that *Moringa* leaves have high nutritional values and contain large amounts of calcium (Ca) recorded (492.5-1819.5 ppm dry weight).

In addition, MON leaves recorded the highest amount of Ca (1819.5 ppm dry matter), followed by MOS, MPG, MSA and MSB recorded (1529, 847.2, 578 and 492.5 ppm dry weight) respectively.

These results were more than the result of the previous studies of Teixeira *et al.,* (2014) for *Moringa* leaves recorded (30 ppm for calcium), and lower than the result of previous studies of the predominant mineral elements in the leaves powder according to ICP-MS were Ca (2016.5–2620.5 mg/100 g) and Mg (322.5–340.6 mg/100 g), (Valdez-Solana *et al.,* 2015). These results is in agreement with 22.4 mg/g calcium (Nambiar, 2006).

**Table 8. Elements analysis of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves.**

| ***Moringa Spp.*** | **Elements in *Moringa* Leaves** | | | | |
|---|---|---|---|---|---|
| | **Ca (ppm)** | **Mg (ppm)** | **Fe (ppm)** | **Zn (ppp)** | **Cu (ppp)** |
| **MSB** | $492.5^{e}\pm1.74$ | $142^{e}\pm1.10$ | $3.67^{c}\pm0.11$ | $146.8^{b}\pm2.21$ | $315.1^{b}\pm3.31$ |
| **MSA** | $578.0^{d}\pm2.03$ | $148^{d}\pm1.42$ | $8.56^{b}\pm0.12$ | $178.1^{a}\pm3.21$ | $255.1^{d}\pm2.13$ |
| **MON** | $1819.5^{a}\pm1.01$ | $169^{a}\pm2.53$ | $8.72^{b}\pm0.22$ | $162.5^{a}\pm2.14$ | $270.1^{c}\pm4.31$ |
| **MOS** | $1529.0^{b}\pm2.31$ | $156^{c}\pm1.24$ | $44.6^{a}\pm0.42$ | $2.9^{d}\pm0.21$ | $805.0^{a}\pm3.56$ |
| **MPG** | $847.2^{c}\pm4.36$ | $163^{b}\pm1.31$ | $4.15^{c}\pm0.12$ | $28.2^{c}\pm1.11$ | $90.1^{e}\pm1.71$ |
| **LSD(0.05)** | **18.4** | **6.5** | **1.1** | **8.1** | **5.1** |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

Narrow range of Magnesium (Mg) values was recorded (142-169 ppm dry weight) for leaves. On the other hand, MON leaves recorded the highest significant amount of Mg (169 ppm dry weight), followed by MPG, MOS, MSA and MSB recorded (163, 156, 148 and 142 ppm dry weight) respectively.

The values of Fe amount recorded range (3.67-44.55 ppm dry weight) for leaves. In addition, MOS recorded the highest significant amount of Fe (44.55-ppm dry weight) for leaves, in agreement with 0.26 mg/g Fe, 22.4 mg/g calcium, 6.3 mg/g P. (Nambiar, 2006).

This result is of special interest which is lower than previous studies of (1031 ppm iron), Teixeira *et al.,* (2014).

The values of Zn amount recorded range (2.9-178.1 ppp dry weight) for leaves. On the other hand, MSA recorded the highest amount of Zn (178.1 ppp dry weight) for leaves. Also, MON and MSB came after MSA recorded high significat amount of Zn for leaves (162.5 and 146.8 ppp dry weight) respectively.

## 4.6. The elements analysis of seeds for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

The data presented in table (9) showed that *Moringa* seeds have high nutritional values and contain large and significat amounts of calcium (Ca) recorded 520.8-1994 ppm dry weight for *Moringa* seeds.

MON seeds recorded the highest significat amount of Ca 1994 ppm dry matter, followed by MSA, MOS, MSB and MPG recorded 1865, 1279, 1206 and 520.8 ppm dry matter, respectively.

Narrow range of Magnesium (Mg) values was 124-154 ppm dry weight for seeds. On the other hand, MOS seeds recorded the highest significant amount of Mg 154 ppm dry weight followed by MSB and MSA in a similar result recorded 153 and 153 ppm dry weight respectively, followed by MON and MPG recorded 147 and 124 ppm dry weight, respectively.

The values of Fe amount recorded range 25.17-62.45 ppm dry weight for seeds. MON recorded the highest significant amount of Fe 62.45 ppm dry weight for seeds. On the other hand, there were similar results for the amount of Fe in the couple of MSA and MOS seeds recorded 36.4 and 35.11 ppm dry weight respectively.

In addition, the couple of MSB and MPG seeds recorded similar results for Fe 28.71 and 25.17 ppm dry weight, respectively. This result is of special interest which is lower than previous studies of 1031 ppm iron, (Teixeira *et al.,* 2014). The values of Zn amount recorded range (1.1-4.8 ppp) for seeds. Although, MON recorded the highest significant amount of Zn for seeds (7.1 ppp dry weight), these amount is very small compared with the Zn amount in leaves. The values of Cu amount recorded range (90-805 ppp dry weight) for leaves and (1.23-1070 ppp) for seeds.

**Table 9. Elements analysis of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* seeds.**

| *Moringa Spp.* | Elements in *Moringa* seeds | | | | |
|---|---|---|---|---|---|
| | **Ca (ppm)** | **Mg (ppm)** | **Fe (ppm)** | **Zn (ppp)** | **Cu (ppp)** |
| **MSB** | $1206^{d}$±2.91 | $153^{a}$±3.93 | $28.71^{c}$±1.36 | $4.80^{b}$±1.73 | $1033.31^{b}$±2.96 |
| **MSA** | $1865^{b}$±4.41 | $153^{a}$±5.03 | $36.4^{b}$±2.00 | $3.27^{c}$±1.20 | $123.20^{e}$±1.45 |
| **MON** | $1994^{a}$±5.69 | $147^{b}$±4.04 | $62.45^{a}$±1.20 | $7.17^{a}$±1.45 | $1071.13^{a}$±4.18 |
| **MOS** | $1279^{c}$±3.84 | $154^{a}$±2.60 | $35.11^{b}$±0.86 | $1.17^{e}$±1.45 | $140.32^{c}$±8.95 |
| **MPG** | $520.8^{e}$±2.39 | $124^{c}$±2.84 | $25.17^{d}$±1.16 | $1.93^{d}$±1.20 | $84.31^{d}$±6.98 |
| **LSD(0.05)** | **21.5** | **8.3** | **3.4** | **0.11** | **10.12** |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

On the other hand, MON recorded the highest amount of Cu (1070 ppp dry weight) for seeds. In addition, MSB came after MON recorded high amount of Cu for seeds (1030 ppp dry weight). On the other hand, MOS recorded the highest significant amount of Cu (805 ppp dry weight) for leaves.

In addition, MSB and MSA came after MOS recorded medium amount of Cu for leaves 315 and 255 ppp dry weight, respectively.

## 4.7. Free fatty acid percentage, peroxide, iodine and saponification values for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* seeds oil.

The data presented in table 10 illustrated by figures (14, 15, 16, and 17) showed that, the free fatty acids percentage recorded narrow range (0.32-0.8%). MPG oil recorded the highest significant percentage of free fatty acids (0.8 %) followed by MSB and MOS oils in a similar result recorded (0.7 and 0.7 %) respectively, followed by MSA and MON recorded (0.5 and 0.3 %) respectively. The findings from this state are in agreement with Abiodun *et al.,* (2012) and Abdel-Baky and El-Baroty (2013).

Peroxide values whereas recorded 2.27- 5.03 mEq. $O_2$/kg oil, MOS oil recorded the highest peroxide value 5.03 mEq.$O_2$/kg oil followed by MON in a similar result recorded 4.5mEq.$O_2$/kg oil, followed by MSB, MSA and MPG 3.9, 2.7 and 2.3 mEq. $O_2$/kg oil, respectively.

Iodine values recorded different results with narrow range 65.34-70.07, MON oil recorded the highest iodine value (70.1), followed by MPG similar result of iodine value recorded (69.3) followed by MSB, MSA and MOS recorded similar result 67.1, 65.8 and 65.3, respectively.

On the other hand, Saponification values recorded mostly similar results with narrow range 175.7-192.3 mEq. KOH/kg oil.

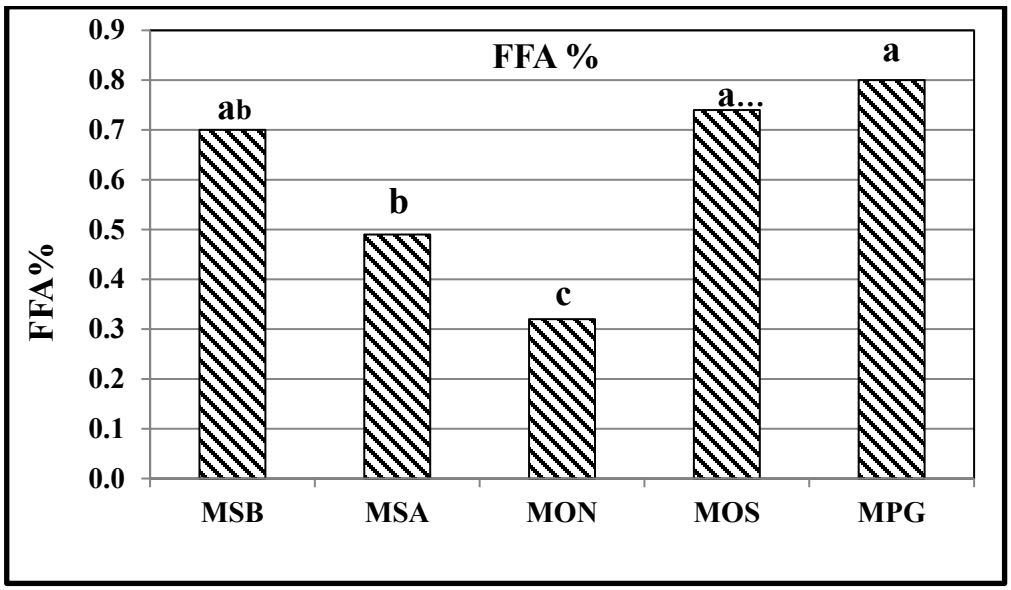



**Figure 14. Free fatty acid percentage of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

MSB oil recorded the highest value 192 mg KOH/g oil, followed by MSA in a similar result of saponification values recorded 189.7 mg KOH/g oil followed by MPG, MON and MOS in a similar result recorded 180.7, 177.7 and 175.7 mg KOH/g oil, respectively.

**Table 10. Free fatty acid percentages, peroxide, iodine and saponification values of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* seeds oil.**

| *Moringa Sp.* | FFA% | P.V (mEq.$O_2$/kgOil) | Iodine Value | S.V (mgKOH/gOil) |
|---|---|---|---|---|
| **MSB** | $0.70^{ab}\pm0.00$ | $3.93^{b}\pm0.18$ | $67.05^{b}\pm0.84$ | $192.33^{a}\pm1.45$ |
| **MSA** | $0.49^{b}\pm0.02$ | $2.65^{c}\pm0.00$ | $65.79^{c}\pm0.66$ | $189.67^{a}\pm1.20$ |
| **MON** | $0.32^{c}\pm0.00$ | $4.50^{ab}\pm0.25$ | $70.07^{a}\pm0.84$ | $177.67^{b}\pm1.45$ |
| **MOS** | $0.74^{ab}\pm0.12$ | $5.03^{a}\pm0.09$ | $65.34^{c}\pm0.00$ | $175.67^{b}\pm0.88$ |
| **MPG** | $0.80^{a}\pm0.01$ | $2.27^{c}\pm0.09$ | $69.31^{ab}\pm1.54$ | $180.67^{b}\pm1.20$ |
| **LSD(0.05)** | **0.17** | **0.47** | **2.1** | **18.1** |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

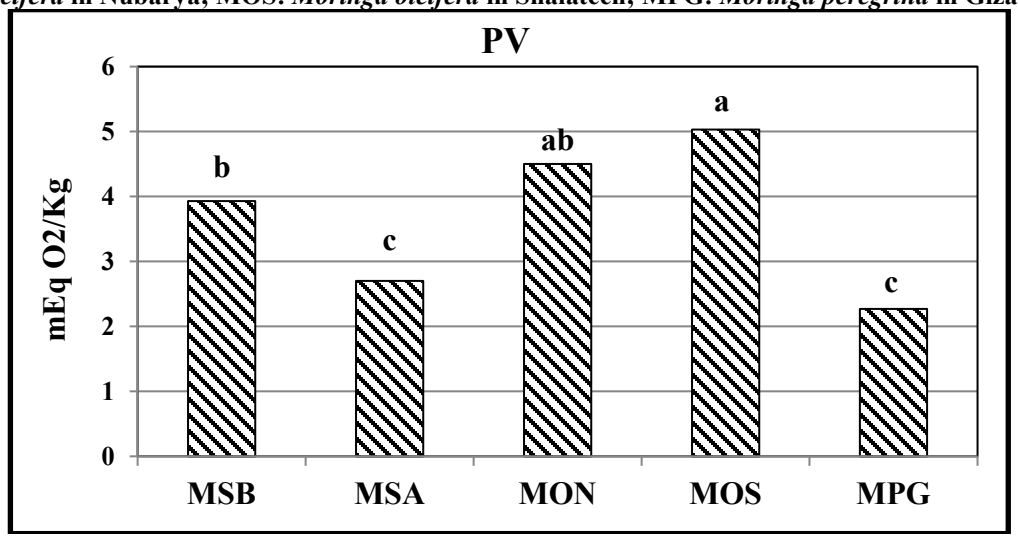



**Figure 15. Peroxide Values of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

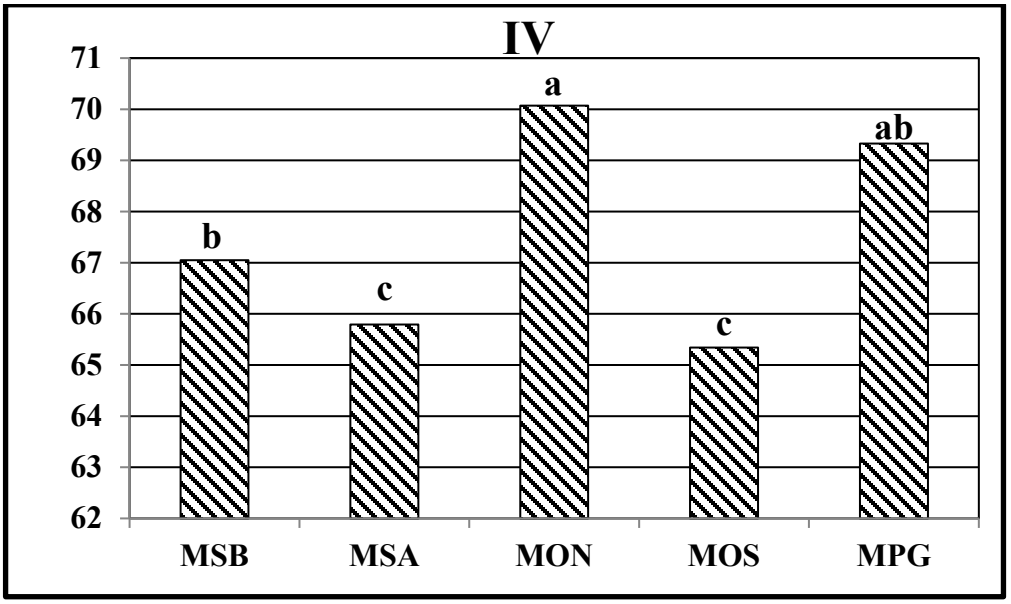



**Figure 16. Iodine Values of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

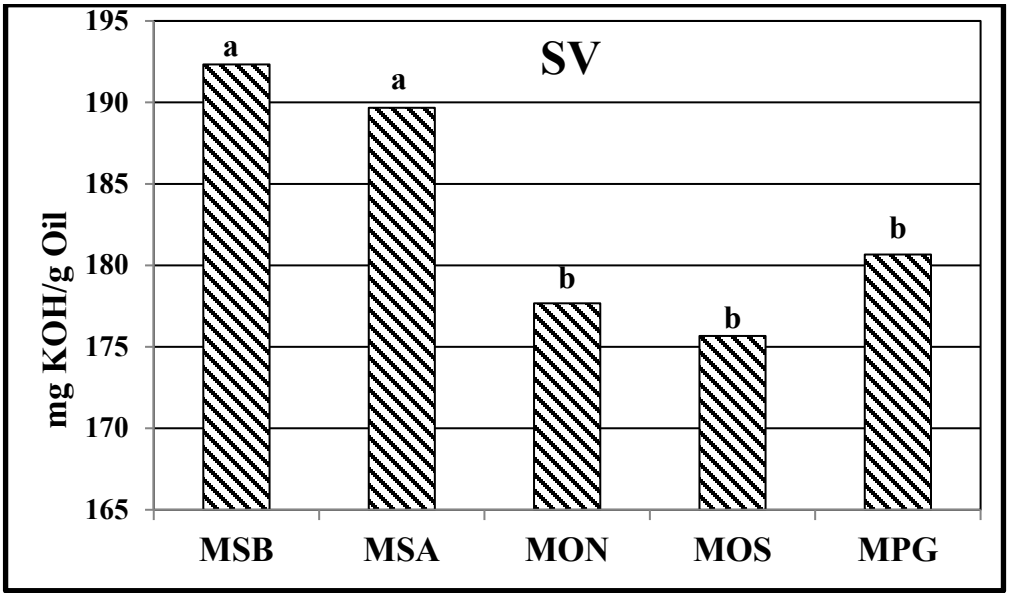



**Figure 17. Saponification values (mg of KOH/g of oil) of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

## 4.8. Fatty acids composition for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* seeds

The fatty acids compositions for *Moringa oleifera, Moringa peregrina and Moringa stenopetala* are presented in table (11). The major saturated fatty acids were palmitic acid (C16:0), the highest value found in MSB recorded (10.31±0.53%), followed by MSA, MON, MOS and MPG recorded similar result (7.28±0.53, 5.93±0.53, 6.02±0.53 and 7.78±0.53%) respectively.

Stearic acid (C18:0) came after palmitic acid (C16:0), the highest value found in MOS, recorded (7.27±0.29 %), followed by MON and MSA in a similar result recorded (5.31±0.29 and 4.2±0.29 %) respectively, followed by MSB and MPG in a similar result recorded (3.49±0.29 and 3.5±0.29%) respectively.

Behenic acid (C22:0) came after Stearic acid (C18:0), the highest value found in MSA, MON and MOS in a similar result recorded (5.02±0.28, 5.94±0.28 and 5.46±0.28 %) respectively, followed by MSB and MPG similar result recorded (3.64±0.49 and 2.56±0.28%) respectively.

The main unsaturated fatty acid is Oleic acid (C18:1ω9). Oleic acid is less reactive and more heat stable, because it contains only one double bond and recorded the highest significant value of Mono-unsaturated fatty acids, MPG oil recorded the highest significant value (66.4±0.3 %), followed by MSB and MSA in similar result recorded (62.4±0.3 and 62.2±0.3 %) respectively, followed by MON and MOS in similar result recorded (60.5±0.3 and 60.4±0.3 %) respectively.

**Table 11. Fatty acids composition of seeds oil extracted from *Moringa oleifera*, *Moringa peregrina* and *Moringa stenopetala***

| | *Moringa Spp.* | | | | | |
|---|---|---|---|---|---|---|
| **Component** | **MSB** | **MSA** | **MON** | **MOS** | **MPG** | **LSD(0.05)** |
| **C16:0** | 10.31$^{a}$ ± 0.5 | 7.28$^{b}$ ± 0.5 | 5.93$^{b}$ ± 0.5 | 6.02$^{b}$ ± 0.5 | 7.78$^{b}$ ± 0.5 | **1.77** |
| **C18:0** | 3.49$^{c}$ ± 0.3 | 4.20$^{bc}$ ± 0.3 | 5.31$^{b}$ ± 0.3 | 7.26$^{a}$ ± 0.3 | 3.50$^{c}$ ± 0.3 | **0.93** |
| **C22:0** | 3.64$^{b}$ ± 0.5 | 5.02$^{a}$ ± 0.3 | 5.94$^{a}$ ± 0.6 | 5.46$^{a}$ ± 0.2 | 2.56$^{b}$ ± 0.3 | **0.79** |
| **C16:1n9** | 1.62$^{b}$ ± 0.1 | 1.40$^{bc}$ ± 0.1 | 1.13$^{c}$ ± 0.1 | 1.15$^{c}$ ± 0.1 | 1.94$^{a}$ ± 0.1 | **0.21** |
| **C16:1n7** | 1.01$^{a}$ ± 0.1 | 0.76$^{ab}$ ± 0.1 | 0.53$^{b}$ ± 0.1 | 0.63$^{b}$ ± 0.1 | 0.78$^{ab}$ ± 0.1 | **0.23** |
| **C18:1n9** | 62.0$^{b}$ ± 0.3 | 62.2$^{b}$ ± 0.3 | 60.5$^{c}$ ± 0.3 | 60.4$^{c}$ ± 0.3 | 66.4$^{a}$ ± 0.3 | **0.93** |
| **C18:1n7** | 3.76$^{c}$ ± 0.1 | 5.10$^{a}$ ± 0.1 | 5.23$^{a}$ ± 0.1 | 4.45$^{b}$ ± 0.1 | 4.34$^{b}$ ± 0.1 | **0.32** |
| **C18:1n5** | 0.60$^{b}$ ± 0.0 | 0.64$^{b}$ ± 0.0 | 0.74$^{b}$ ± 0.0 | 0.99$^{a}$ ± 0.0 | 0.60$^{b}$ ± 0.0 | **0.11** |
| **C20:1n11** | 1.86$^{a}$ ± 0.1 | 2.12$^{a}$ ± 0.1 | 2.06$^{a}$ ± 0.1 | 1.55$^{b}$ ± 0.1 | 1.40$^{b}$ ± 0.1 | **0.20** |
| **C20:1n9** | 1.68$^{a}$ ± 0.2 | 0.60$^{b}$ ± 0.2 | 0.32$^{b}$ ± 0.2 | 0.43$^{b}$ ± 0.2 | 0.42$^{b}$ ± 0.2 | **0.55** |
| **C22:1n9** | 0.28$^{b}$ ± 0.0 | 0.39$^{a}$ ± 0.0 | 0.49$^{a}$ ± 0.0 | 0.14$^{c}$ ± 0.0 | 0.20$^{bc}$ ± 0.0 | **0.07** |
| **C18:2n6** | 5.39$^{a}$ ± 0.2 | 5.93$^{a}$ ± 0.2 | 5.76$^{a}$ ± 0.2 | 6.00$^{a}$ ± 0.2 | 6.42$^{a}$ ± 0.2 | **0.71** |
| **C18:2n4** | 0.49$^{a}$ ± 0.0 | 0.48$^{a}$ ± 0.0 | 0.51$^{a}$ ± 0.0 | 0.46$^{a}$ ± 0.0 | 0.43$^{a}$ ± 0.0 | **0.08** |
| **C18:2n7** | 0.52$^{bc}$ ± 0.0 | 0.55$^{b}$ ± 0.0 | 1.29$^{a}$ ± 0.0 | 0.56$^{b}$ ± 0.0 | 0.49$^{c}$ ± 0.0 | **0.04** |
| **C18:4n3** | 2.31$^{cd}$ ± 0.2 | 2.77$^{bc}$ ± 0.2 | 3.28$^{b}$ ± 0.2 | 4.21$^{a}$ ± 0.2 | 1.8$^{d}$ ± 0.1 | **0.48** |
| **W3** | 2.31$^{cd}$ ± 0.2 | 2.77$^{bc}$ ± 0.2 | 3.28$^{b}$ ± 0.2 | 4.21$^{a}$ ± 0.2 | 1.8$^{d}$ ± 0.1 | **0.46** |
| **W6** | 6.41$^{b}$± 0.2 | 6.97$^{ab}$ ± 0.2 | 7.56$^{a}$ ± 0.2 | 7.01$^{ab}$ ± 0.2 | 7.3$^{ab}$ ± 0.2 | **0.64** |
| **W3/W6** | 2.8$^{b}$± 0.2 | 2.6$^{b}$ ± 0.2 | 2.31$^{bc}$ ± 0.2 | 1.67$^{c}$ ± 0.2 | 3.9$^{a}$ ± 0.2 | **0.62** |
| **TSFA** | 17.4$^{b}$± 0.1 | 16.5$^{c}$ ± 0.1 | 17.2$^{b}$ ± 0.1 | 18.7$^{a}$ ± 0.1 | 13.8$^{d}$ ± 0.1 | **0.21** |
| **PUSFA** | 72.8$^{b}$ ± 0.3 | 73.2$^{b}$± 0.3 | 71.0$^{c}$ ± 0.3 | 69.7$^{c}$ ± 0.3 | 76.0$^{a}$ ± 0.3 | **0.84** |
| **C18:2/C16:0** | 0.62$^{c}$± 0.1 | 1.0$^{ab}$ ± 0.1 | 1.28$^{a}$ ± 0.1 | 1.17$^{ab}$ ± 0.1 | 0.94$^{b}$± 0.1 | **0.21** |
| **PUSFA/SFA** | 0.50$^{c}$ ± 0.0 | 0.59$^{b}$ ± 0.0 | 0.63$^{ab}$ ± 0.0 | 0.60$^{b}$ ± 0.0 | 0.66$^{a}$ ± 0.0 | **0.04** |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

The data observed that Vaccinic fatty acid (C18:1ω7) followed by small amount of Eicosenoic (C20:1ω11), (C16:1ω9), Eicosenoic (C20:1ω9), Palmitoleic acids (C16:1ω7), (C18:1ω5) and smallest amount of Docosenoic (C22:1ω9), recorded ranges (5.23±0.1-3.76±0.1, 2.12±0.1-1.4±0.1, 1.94±0.07-1.13±0.07, 1.68±0.2-0.32±0.2, 1.01±0.07-0.53±0.07, 0.99±0.03-0.6±0.03 and 0.49-0.14 %) respectively.

For these compounds, Anwar *et al*. (2006) have found similar results; while Abdulkarim *et al*. (2005) have reported different results. Differences observed between results can be attributed to geographical, soil composition, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method used.

The high percentage of oleic acid (monounsaturated fatty acid) in the oil makes it desirable in terms of nutrition and high stability cooking and frying oil, (Abdulkarim *et al.,* 2005; Anwar *et al.,* 2006).

A higher intake of oleic acid is associated with decreased risk of coronary heart disease caused by high cholesterol level in blood (Corbett, 2003). Omega (6) recorded a significant amounts, Linoleic fatty acid (C18:2ω6) is a highly sensitive to oxidation and contains two double bands showed similar result with no significant difference among them recorded range (6.42-5.39 %).

Also the fatty acid (C18:2ω4) showed a similar result with no significant difference among them recorded range (0.51-0.43 %). On the other hand, the fatty acid (C18:2ω7) showed a significant differences recorded range (1.29±0.01-0.49±0.01 %).

Omega (3) showed a significant amounts, Octadecatetraenoic fatty acid (C18:4ω3) recorded range (4.21±0.15-1.84±0.15 %).

Total unsaturated fatty acids (TUFA) showed a significant differences, MPG oil recorded the highest significant percentage (76.03±0.27%), followed by MSB and MSA in similar result recorded (73.21±0.27 and 72.79±0.27%) respectively, followed by MON and MOS in similar result recorded (71.02±0.27 and 69.74±0.27%) respectively as shown in Fig. (20).

The seeds oil can also be used as a natural source of behenic acid, which has been used as an oil structuring and solidifying agent in margarine, shortening, and foods containing semi-solid and solid fats, eliminating the need to hydrogenate the oil, (Foidl *et al.,* 2001).

*Moringa oleifera* oil appears to be a potentially valuable and might be an acceptable substitute for high-oleic oils like olive and high-oleic sunflower oils as our dietary fats and it could be used for various commodities of commercial attributes, (Anwar *et al.,* 2006).

On the other hand, Total Saturated Fatty Acids (TSFA) showed a significant differences, MOS oil recorded the highest significant percentage (18.74±0.07%), followed by MSB, MON and MSA in similar result recorded (17.44±0.07, 17.18±0.07 and 16.5±0.07) respectively, followed by MPG recorded (13.84±0.07).

In addition, PUFA and TSFA showed complementary relationship as shown in figures. (18, 19 and 20).

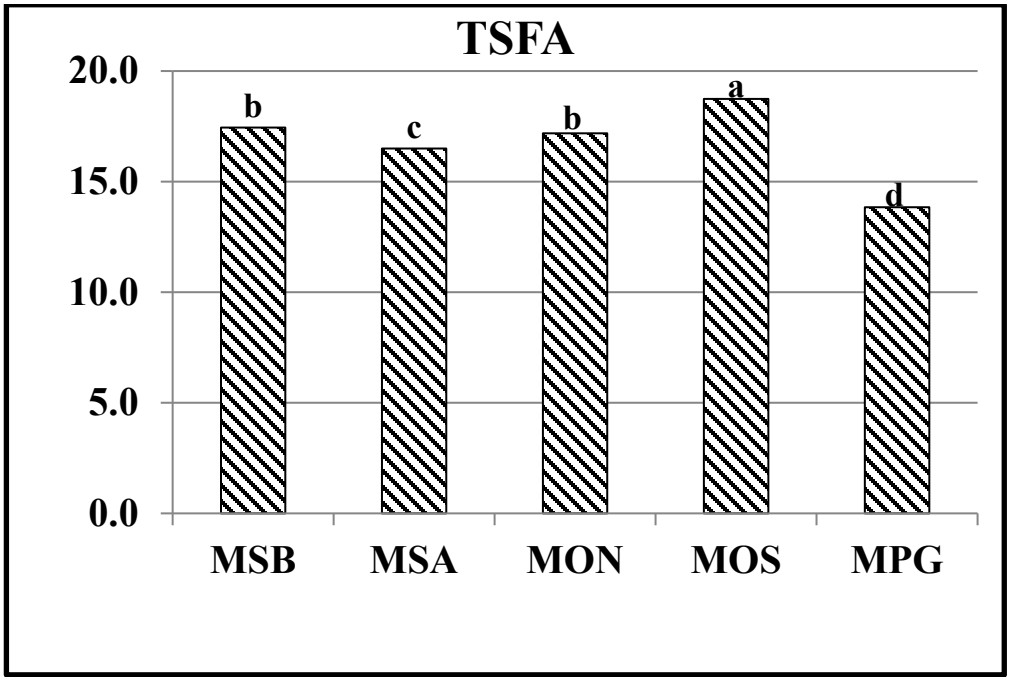



**Figure 18. TUSFA of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

In addition, W6/W3 ratio showed a significant differences, MPG oil showed the highest significant ratio recorded (3.98±0.2), followed by MSB, MSA and MON in similar significant result recorded (2.78±0.2, 2.61±0.2 and 2.31±0.2) respectively, followed by MOS recorded (1.67±0.2).

On the other hand, PUFA/SFA showed significant differences, MPG recorded the highest significant ratio (0.66±0.1), followed by MON in a similar significant result (0.63±0.1), followed by MOS and MSA in a similar result recorded (0.6o±0.1 and 0.59±0.1) and followed by MSB recorded (0.50±0.1).

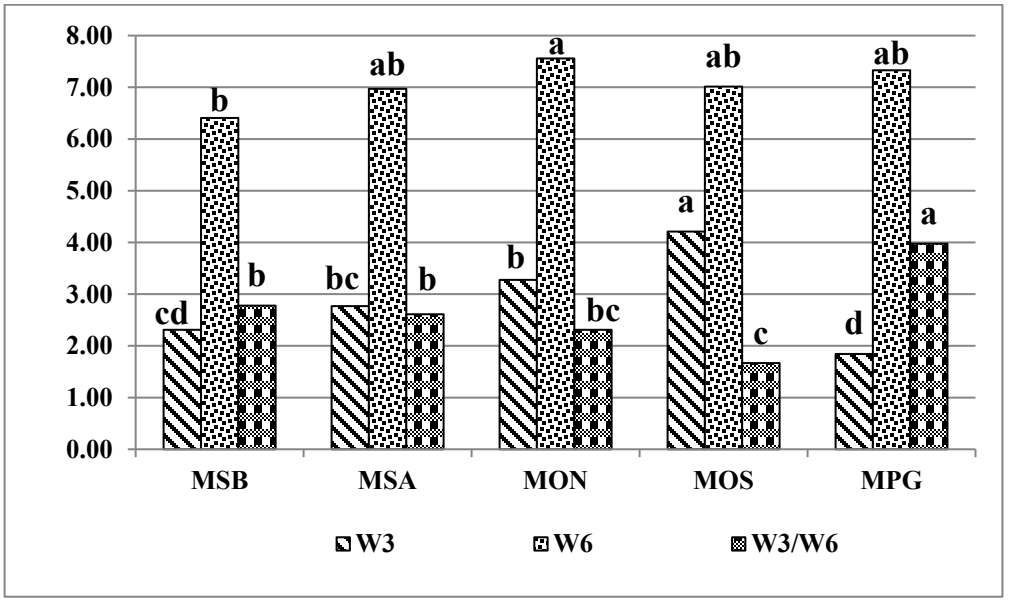



**Figure 19. W3, W6 and W3/W6 Ratio of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

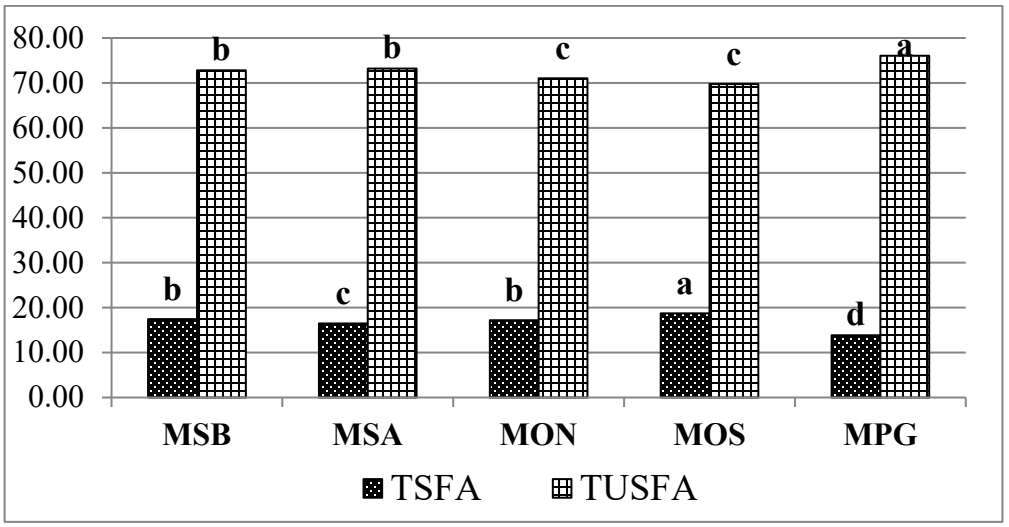



**Figure 20. TSFA and PUSFA of *Moringa Stenopetala, Moringa Oleifera* and *Moringa Peregrina* seeds oil. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

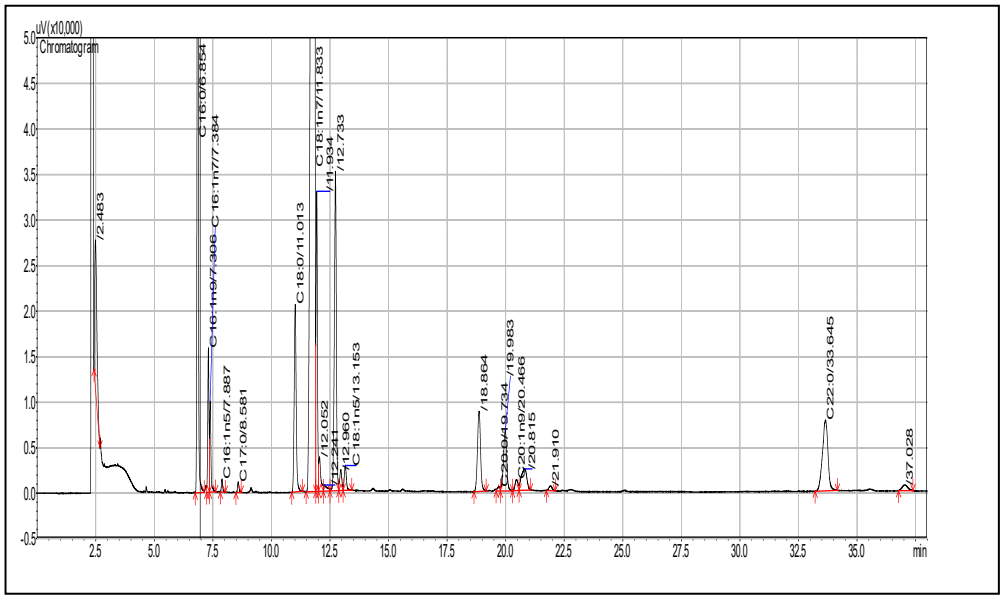



**Figure 21. Chromatogram of fatty acid profile for *Moringa stenopetala* (MSB) in Belbis, Sharkia, Egypt, 2014.**

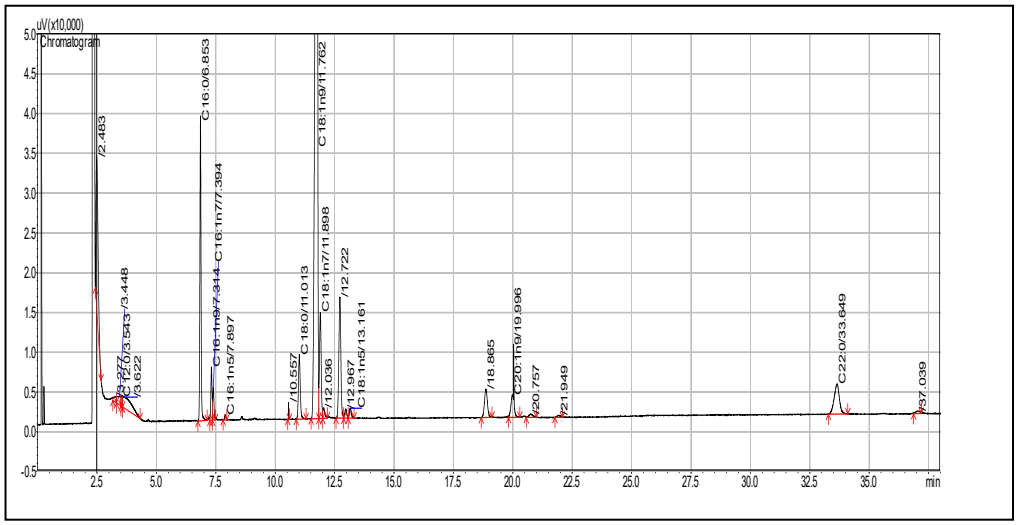



**Figure 22. Chromatogram of fatty acid profile for *Moringa stenopetala* (MSA) in Aswan, Egypt, 2014.**

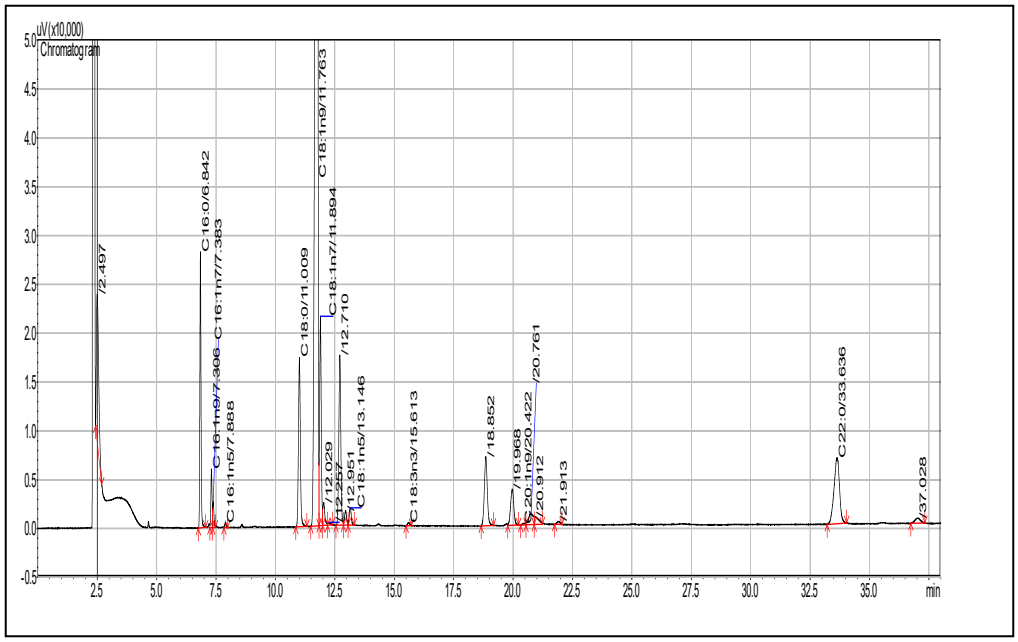



**Figure 23. Chromatogram of fatty acid profile for *Moringa olifiera* (MON) in Nubaraia, Egypt, 2014**

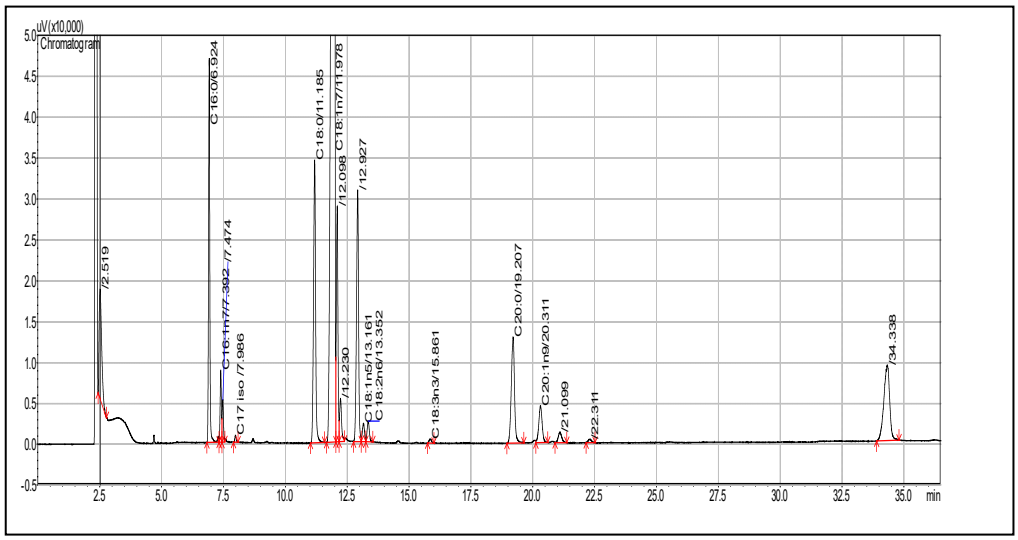



**Figure 24. Chromatogram of fatty acid profile for *Moringa olifiera* (MOS) in, Shalateen, Egypt, 2014**

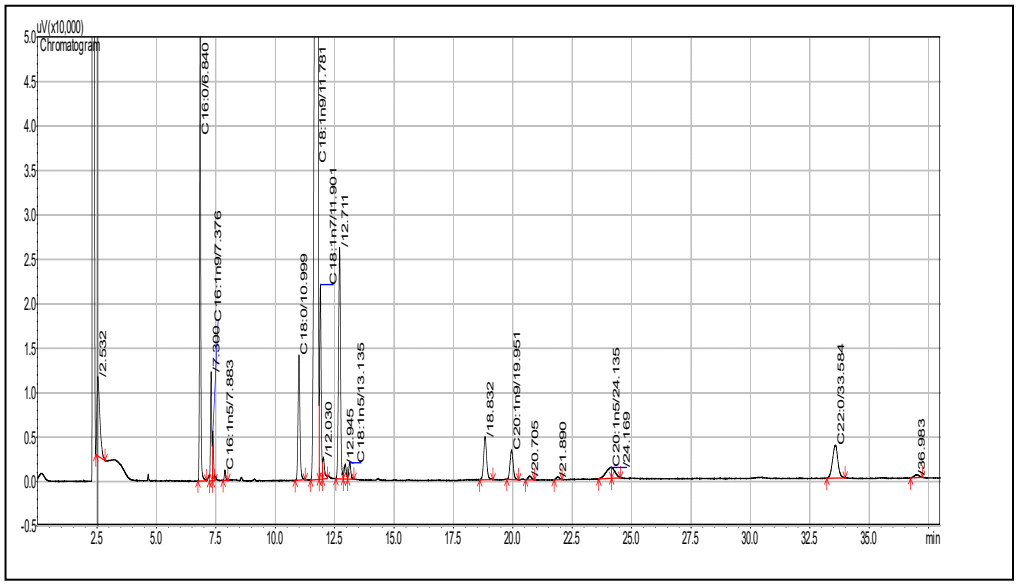



**Figure 25. Chromatogram of fatty acid profile for *Moringa peregrina* (MPG) in Giza, Egypt, 2014**

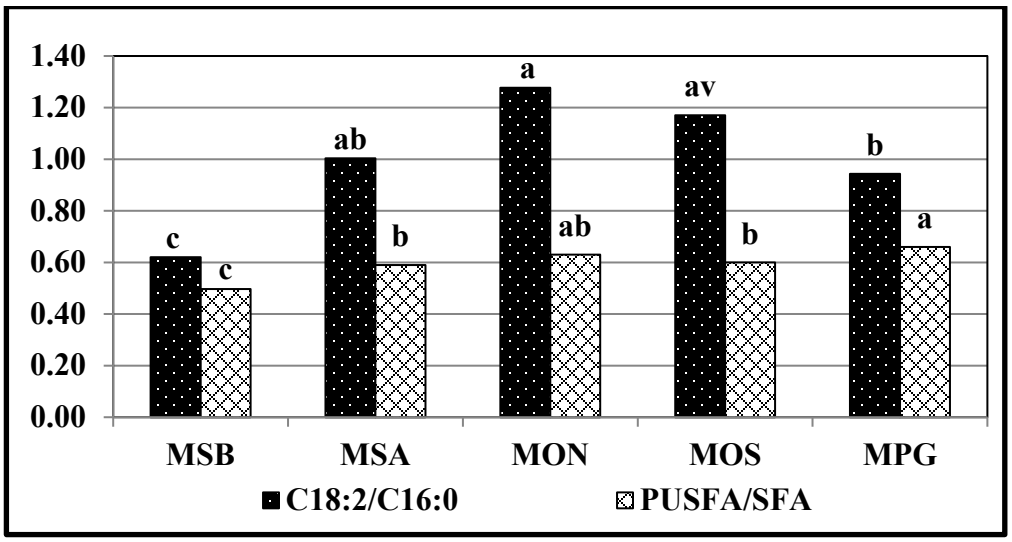



**Figure 26. C18:2/C16:0 and PUSFA/SFA of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

## 4.9. Amino acids composition of leaves for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

The Amino Acids compositions were determined and the data obtained are outlined in table (12) illustrated by figure (27). The data illustrated that *Moringa* leaves recorded significant increases for total summation of amino acids percentage (9.85-24.87%), in agreement with Kayi, (2013) and Nilanjana, (2014). On the other hand, amino acids /protein ratio recorded high percentage range (80.08-85.45%).

The data observed that Glutamic, Aspartic, Leucine, Phenylalanine, Alanine and Arginine acids were the most predominant A. A recorded (1.17-3.47; 1.04-2.9; 0.83-2.16; 0.59-1.63; 0.61-1.54 and 0.68-1.53) respectively.

These recorded results were in agreement with recorded data for Leucine, Phenylalanine and Arginine acids were recorded (1.6-2.1; 1-1.64 and 1.3-1.9%) respectively, according to Kayi, (2013).

Also in agreement with data for Leucine, Phenylalanine and Arginine acids were recorded (1.9; 1.39 and 1.33%) respectively, according to Nilanjana, (2014), followed by Valine, Lysine, Glycine, Isoleucine, Threonine and Proline acids were the middle predominant A.A recorded (0.59-1.49; 0.65-1.46; 0.55-1.27; 0.43-1.18; 0.46-1.16 and 0.49-1.12) respectively, in agreement with data for Valine, Lysine, Isoleucine, and Threonine acids recorded (1.1-1.48; 0.92-1.6; 0.78-1.19 and 0.71-1.36%) respectively according to Kayi, (2013).

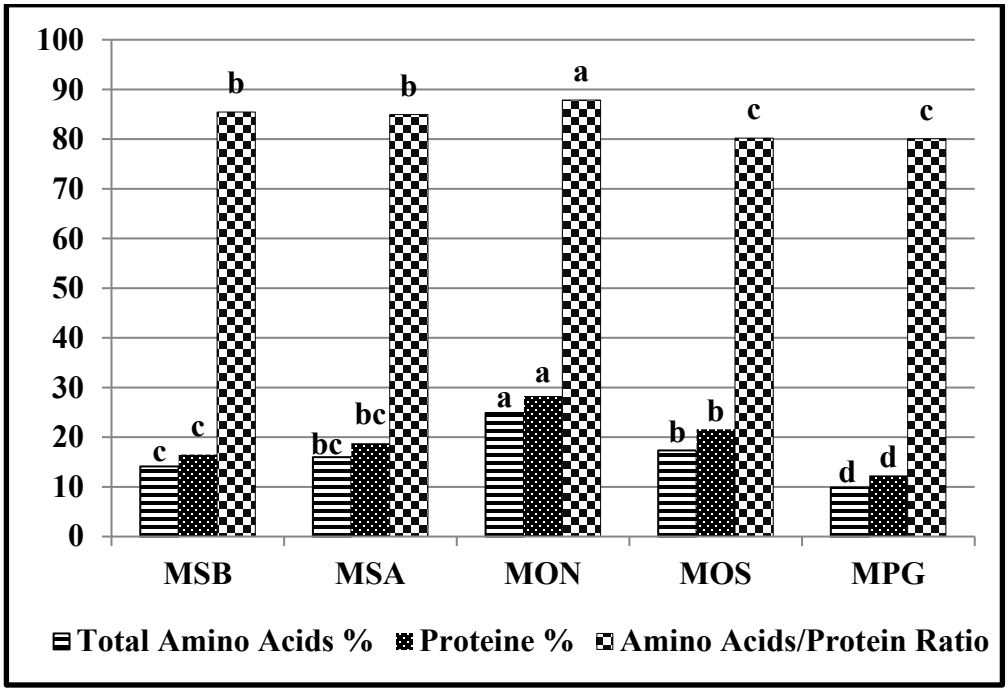



**Figure 27. Total amino acid and protein percentages of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

Also in agreement with data for Valine, Lysine, Isoleucine, and Threonine acids recorded (1.06; 1.3; 0.83 and 1.19%) respectively according to Nilanjana, (2014), followed by Serine, Tyrosine, Histidine, Cyctine and Methionine acids, were the lowest predominant A. A recorded (0.54-1.07; 0.44-1.07; 0.24-0.76; 0.32-0.69 and 0.22-0.37) respectively, in agreement with data for Tyrosine, Histidine, Cyctine and Methionine acids recorded (0.83-2.65; 0.38-0.72; 0.01-0.39 and 0.23-0.35%) respectively according to Kayi, (2013), also in agreement with data for Histidine and Methionine acids recorded (0.61 and 0.35%) respectively according to Nilanjana, (2014).

**Table 12. Amino acid analysis of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves.**

| Chemical Compound | Percentage of chemical compounds | | | | |
|---|---|---|---|---|---|
| | MSB | MSA | MON | MOS | MPG |
| **Aspartic** | 1.50±0.02 | 2.13±0.03 | 2.9±0.04 | 2.01±0.06 | 1.04±0.01 |
| **Threonine** | 0.71±0.01 | 0.75±0.01 | 1.16±0.02 | 0.78±0.03 | 0.46±0.01 |
| **Serine** | 0.75±0.01 | 0.72±0.01 | 1.07±0.01 | 0.84±0.03 | 0.54±0.01 |
| **Glutamic** | 1.76±0.03 | 1.88±0.02 | 3.47±0.03 | 2.56±0.04 | 1.17±0.02 |
| **Glycine** | 0.81±0.01 | 0.85±0.01 | 1.27±0.02 | 0.89±0.01 | 0.55±0.01 |
| **Alanine** | 0.88±0.02 | 1.01±0.01 | 1.54±0.02 | 1.11±0.01 | 0.61±0.01 |
| **Valine** | 0.83±0.02 | 1.00±0.01 | 1.49±0.02 | 1.06±0.02 | 0.59±0.01 |
| **Isoleucine** | 0.66±0.01 | 0.70±0.01 | 1.18±0.01 | 0.79±0.02 | 0.43±0.01 |
| **Leucine** | 1.28±0.02 | 1.37±0.02 | 2.16±0.02 | 1.38±0.03 | 0.83±0.02 |
| **Tyrosine** | 0.7±0.01 | 0.73±0.01 | 1.07±0.01 | 0.76±0.02 | 0.44±0.01 |
| **Ph. Alanine** | 0.94±0.02 | 1.17±0.02 | 1.63±0.02 | 1.21±0.02 | 0.59±0.01 |
| **Histidine** | 0.41±0.01 | 0.49±0.01 | 0.76±0.01 | 0.52±0.01 | 0.24±0.01 |
| **Lysine** | 0.93±0.02 | 0.89±0.01 | 1.46±0.01 | 0.91±0.01 | 0.65±0.01 |
| **Arginine** | 0.79±0.01 | 1.02±0.01 | 1.53±0.02 | 1.12±0.02 | 0.68±0.02 |
| **Proline** | 0.61±0.01 | 0.67±0.02 | 1.12±0.01 | 0.77±0.01 | 0.49±0.01 |
| **Cyctine** | 0.32±0.01 | 0.34±0.01 | 0.69±0.01 | 0.36±0.01 | 0.32±0.01 |
| **Methionine** | 0.22±0.01 | 0.25±0.01 | 0.37±0.01 | 0.25±0.01 | 0.22±0.01 |
| **Total A. A** | **14.1±0.11** | **15.97±0.12** | **24.87±0.14** | **17.32±0.15** | **9.85±0.11** |
| **Protein** | **16.5±0.13** | **18.8±0.14** | **28.3±0.21** | **21.6±0.23** | **12.3±0.12** |
| **A.A/P. Ratio** | **85.45±2.31** | **84.95±2.21** | **87.88±2.34** | **80.19±2.16** | **80.08±2.11** |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

On the other hand, the highest total amino acids values were recorded (24.87%) by MON followed by (17.32%) MOS, also MSA recorded (15.97%), and MSB recorded (14.1%), but the lowest total amino acids values (9.85%) recorded by MPG.As shown in Table (12), the calculated Amino Acid/Protein Ratio recorded high values, MON recorded the highest value (87.88%) followed by MSB, MSA, MOS and MPG recorded (85.45, 84.95, 80.19 and 80.08%) respectively.

According to species the amount of Amino Acids varied and the percentage of protein variable for MPG, MSB, MSA, MOS and MON were (12.3, 16.5, 18.8, 21.6 and 28.3) respectively as illustrated by Fig. (23).

## 4.10. Total phenolic contents and antioxidants scavenging activity on DPPH radicals for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves.

The phenolic contents and antioxidants scavenging activity on DPPH radicals were determined and the data obtained are outlined in table 13 illustrated by figures 28 and 29.

The data illustrated that *Moringa* leaves recorded significant increases for aqueous and ethanolic extracts of dried leaves of *Moringa oleifera* Lam. from different agro climatic regions were examined for radical scavenging capacities and antioxidant activities, all leaf extracts were capable of scavenging free radicals, similar scavenging activities for different solvent extracts of each collection were found for the stable 1,1-diphenyl 2-picrylhydrazyl ($DPPH^{\bullet}$) radical, the antioxidant activities (42.82-62.22%).

Among the five different *Moringa* samples, the ethanolic extracts of MSA leaves showed the highest antioxidant activities, 62.11% these interest result is in agreement with the previous study antioxidant activity range (65.1-66.8%), and is lower than (77.95%), (Abdel-Baky and El-Baroty 2013).

**Table 13. Total phenol contents and scavenging activity on DPPH radicals for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves.**

| *Moringa Spp.* | Total Phenolic Contents mg/g (GAE) | Antioxidant Activity (%) |
|---|---|---|
| **MSB** | **34.2$^{a}$ ± 0.87** | **45.58$^{d}$ ± 0.61** |
| **MSA** | **26.7$^{b}$ ± 0.60** | **62.11$^{a}$ ± 0.88** |
| **MON** | **23.3$^{b}$ ± 0.87** | **59.82$^{b}$ ± 2.60** |
| **MOS** | **23.3$^{b}$ ± 0.63** | **42.82$^{e}$ ± 1.45** |
| **MPG** | **38.3$^{a}$ ± 0.61** | **51.27$^{c}$ ± 2.03** |
| **LSD (0.05)** | **4.72** | **0.21** |

**Phenolic content (lg GAEs/mg extract). GAEs. gallic acid equivalents; AOA% Antioxidant Activity % on DPPH radical. Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

Nonetheless, increasing concentration of all the extracts had significantly increased reducing power, which may in part be responsible for their antioxidant activity. The major bioactive compounds of total phenolic contents (23.3-34.2 mg/g GAE), the highest level for total phenolic contents

recorded by MPG (38.3 mg/g GAE), followed by MSB (34.2mg/g GAE), MSA (26.7 mg/g GAE) and similar result for MON and MOS (23.3 and 23.3 mg/g GAE) were found to be flavonoid groups such as quercetin and kaempferol.

Based on the results obtained, *Moringa* leaves are found to be a potential source of natural antioxidants due to their marked antioxidant activity. Almost all plant secondary metabolites and phenolic compounds are bioactive and widely present in all plant kingdom. The color, sensory qualities, nutritional and antioxidant properties completely depends on phenolic compounds and their derivatives, (Al-Saeedi and Hossain 2015; Alabri *et al.,* 2014 and Hossain *et al.,* 2013).

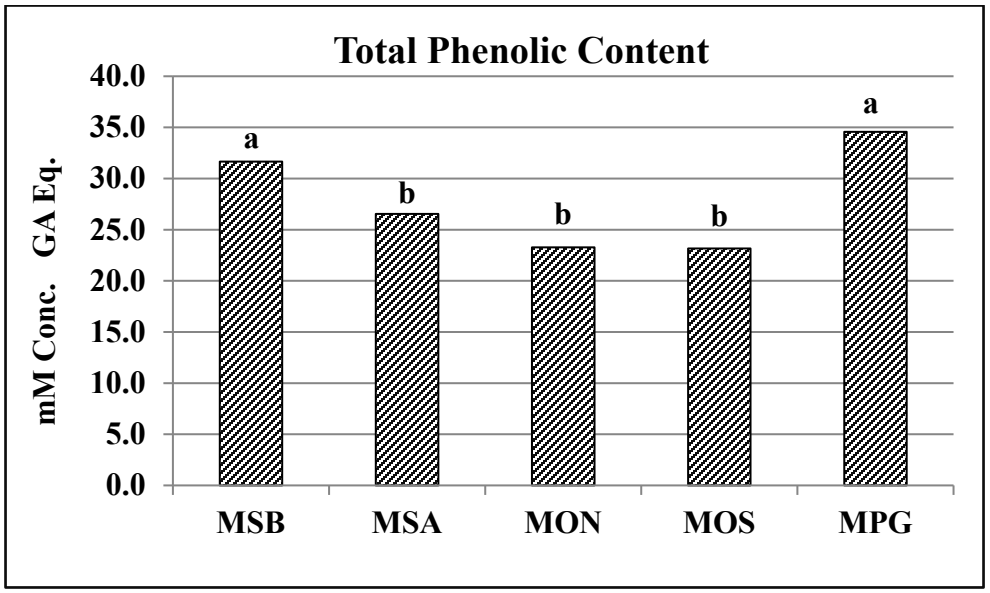



**Figure 28. Total Phenolic Content of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

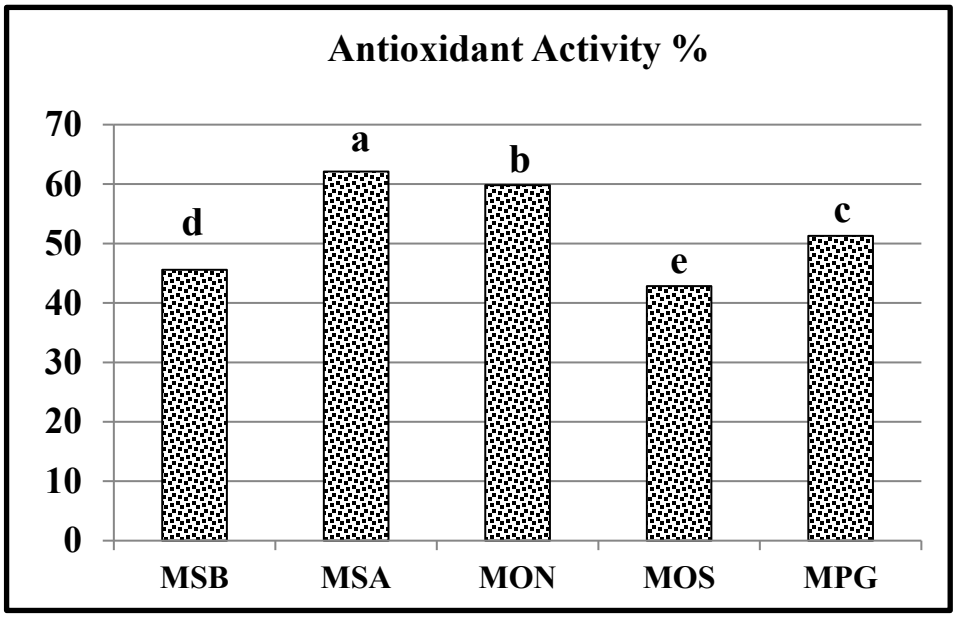



**Figure 29. Antioxidant activity percentage of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

## 4.11. Vitamin (A) analysis for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves

Vitamin (A) analysis for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves were determined and the data obtained are outlined in table (14) illustrated by figure (30).

The data illustrated that *Moringa* leaves also contain high quantities of nutrients (per 100 g fresh weight) vitamin A (5653.9-15851.1µg/100g), the highest significant content recorded by MON (15851.1 µg/100g), followed by MSA (1-9108.7 µg/100g), and MSB (8860.5 µg/100g); MPG (7092.4 µg/100g) and MOS (5653.9 µg/100g), these interest result is in agreement with similar result (7564 IU), (Foidl and Paull, 2008).

**Table 14. Vitamin (A) analysis for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves.**

| *Moringa* spp. | Vitamin A(µg/100g) |
|---|---|
| MSB | $8860.5^{b} \pm 41.52$ |
| MSA | $9108.7^{b} \pm 58.11$ |
| MON | $15851.1^{a} \pm 45.54$ |
| MOS | $5653.9^{d} \pm 31.89$ |
| MPG | $7092.4^{c} \pm 22.52$ |
| LSD (0.05) | 26.5 |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

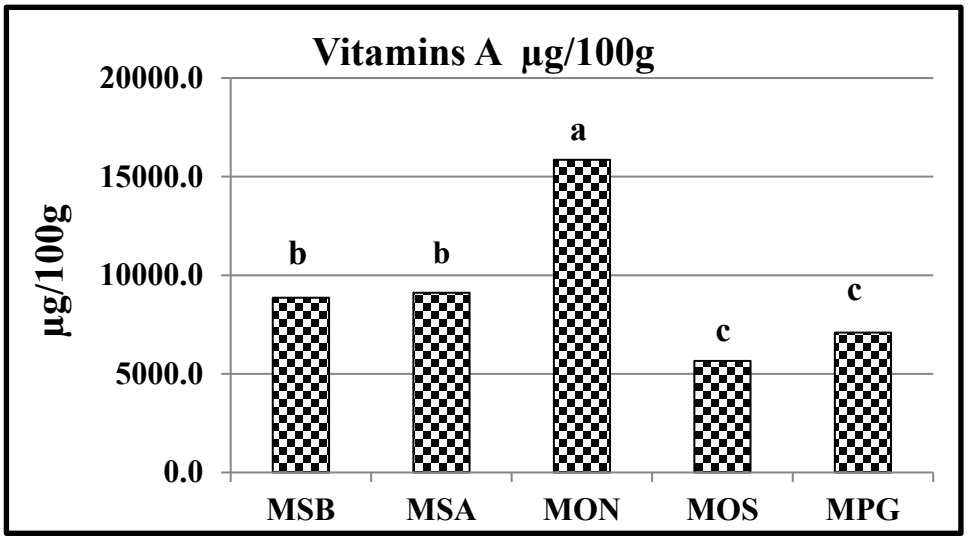



**Figure 30. Vitamin (A) of MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

On the other hand, *Moringa* has the potential to combat vitamin A and other micronutrient deficiencies 40139 µg/100g total carotenoids on fresh weight basis in *Moringa* leaves of which 47.8% or 19210 µg/100g was β-carotene, Ascorbic acid at (6.6 mg/g) on dry weight (Nambiar, 2006).

## 4.12. Vitamin ( C ) analysis for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves

Vitamin (C) analysis for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves were determined and the data obtained are outlined in table (15) illustrated by figure (31).

The data illustrated that *Moringa* leaves also contain high significant quantities of nutrients (per 100 g fresh weight): vitamin C (12.8-399.0 µg/100g), the highest significant content recorded by MSA (399.0µg/100g), followed by MON (249.0µg/100g), and MGP (105.5µg/100g); MSB (51.2µg/100g) and MOS (12.8 µg/100g).

## 4.13. Determination of chemical functional groups for *Moringa oleifera, Moringa peregrina and Moringa stenopetala* by gas chromatography mass.

The data presented in tables (16 and 17) illustrated by figures (32 - 40) showed that, Twenty-eight of chemical volatile and non-volatile compounds and functional groups were determined in *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* by gas chromatography mass.

**Table 15. Vitamin ( C ) analysis for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves.**

| *Moringa* spp. | Vitamin C (µg/100g) |
|---|---|
| MSB | $51.20^{d} \pm 0.58$ |
| MSA | $399.00^{a} \pm 3.18$ |
| MON | $249.00^{b} \pm 3.46$ |
| MOS | $12.80^{e} \pm 1.45$ |
| MPG | $105.50^{c} \pm 2.24$ |
| LSD (0.05) | 26.5 |

**Each value in the table was obtained by calculating the average of the three experiments (Mean±S.E). The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

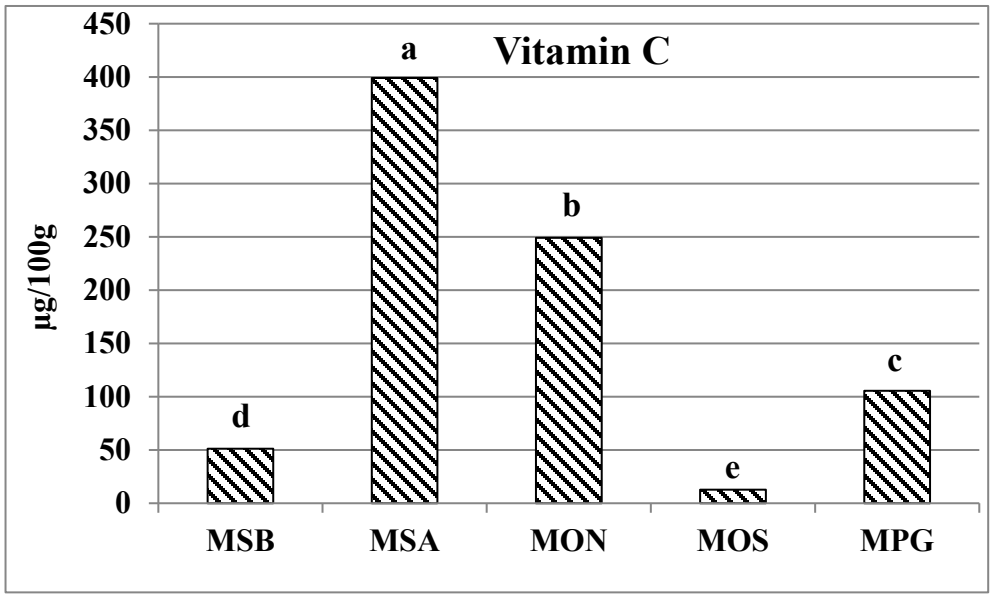



**Figure 31. Vitamin (C) of *Moringa stenopetala, Moringa oleifera* and *Moringa peregrina* leaves, MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON:**

***Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

MSB leaves ethanolic extract contain (11) volatile chemical compounds showed in figure (32) and their structures were showed in figures (37-40).

MSB recorded the highest significant values of 3-Methyl-L-Histidine, Nonadecanoic Acid, Flavone, 5,7-Dimethoxy, Genistin and A-Bisabolol (48.1, 3.4, 3.9, 22.9 and 4.3 %) respectively.

On the other hand, MSA leaves ethanolic extract contain (20) volatile chemical compounds showed in figure (33) and their structures were showed in figures (37-40). MSA recorded the highest values of Glycylglycine, Geranyl Iso-Valerate, Butylphosphonic Acid, Octanoic Acid, Linolenic Acid, 2,6-Di-Tert-Butylhydroquinone and (+)-A-Tocopherol (1.4, 0.4, 1.1, 0.8, 1.8, 2.4 and 5.6 %) respectively.

Also, MON leaves ethanolic extract contain 15 volatile chemical compounds showed in figure (34) and their structures were showed in figures (37-40). MON recorded the highest values of Cysteine, Kaempferol, Cis-13-Eicosenoic Acid, 3, 5-Di-Tert-Butylcatechol and (+)-Nerolidol (29.0, 3.6, 5.4, 0.5 and 0.7 %) respectively.

In addition, MOS leaves ethanolic extract contain 14 volatile chemical compounds showed in figure (35) and their structures were showed in figures (37-40). MOS recorded the highest values of Ascorbic Acid, Permethyl, Quercetin 3',4',7-Trimethyl Ether, Cis-Vaccenic Acid and 24,25-Dihydroxyvitamin D3 ( 10.1, 0.3, 30.6 and 0.9 %) respectively.

**Table 16. Gas chromatography mass analysis for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves ethanolic extract.**

| No. | Compound Name | Compound percentage of *Moringa* leaves ethanolic extract | | | | |
|---|---|---|---|---|---|---|
| | | **MSB** | **MSA** | **MON** | **MOS** | **MPG** |
| 1 | L-Methionine | $6.0^{b}$ | $1.0^{c}$ | $0.0^{d}$ | $0.0^{d}$ | $13.7^{a}$ |
| 2 | Cysteine | $4.4^{d}$ | $11.3^{c}$ | $29.1^{a}$ | $13.8^{b}$ | $2.5^{e}$ |
| 3 | Kaempferol | $0.5^{c}$ | $1.8^{b}$ | $3.6^{a}$ | $1.9^{b}$ | $1.7^{b}$ |
| 4 | 3-Methyl-L-Histidine | $48.1^{a}$ | $20.5^{b}$ | $9.5^{d}$ | $12.5^{c}$ | $7.1^{e}$ |
| 5 | Cis-13-Eicosenoic Acid | $1.6^{d}$ | $2.9^{c}$ | $5.4^{a}$ | $3.9^{b}$ | $0.0^{e}$ |
| 6 | Arachidonic Acid, Ethyl Ester | $3.9^{c}$ | $1.2^{d}$ | $21.4^{b}$ | $3.4^{c}$ | $47.7^{a}$ |
| 7 | Ascorbic Acid, Permethyl | $1.1^{c}$ | $0.6^{d}$ | $0.5^{d}$ | $10.1^{a}$ | $2.1^{b}$ |
| 8 | Nonadecanoic Acid | $3.4^{a}$ | $0.8^{d}$ | $0.9^{d}$ | $1.4^{c}$ | $2.6^{b}$ |
| 9 | Flavone, 5,7-Dimethoxy | $3.9^{a}$ | $0.5^{d}$ | $3.4^{b}$ | $0.6^{d}$ | $2.9^{c}$ |
| 10 | Genistin | $22.9^{a}$ | $18.1^{b}$ | $12.6^{c}$ | $18.7^{b}$ | $3.2^{d}$ |
| 11 | A-Bisabolol | $4.3^{a}$ | $1.9^{b}$ | $0.9^{d}$ | $0.9^{d}$ | $1.5^{c}$ |
| 12 | Glycylglycine | $0.0^{b}$ | $1.4^{a}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ |
| 13 | Geranyl Iso-Valerate | $0.0^{b}$ | $0.4^{a}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ |
| 14 | Quercetin 3',4',7-Trimethyl Ether | $0.0^{c}$ | $0.1^{b}$ | $0.0^{c}$ | $0.3^{a}$ | $0.0^{c}$ |
| 15 | Butylphosphonic Acid | $0.0^{b}$ | $1.1^{a}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ |
| 16 | Octanoic Acid | $0.0^{b}$ | $0.8^{a}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ |
| 17 | Linolenic Acid | $0.0^{b}$ | $11.8^{a}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ |
| 18 | 2,6-Di-Tert-Butylhydroquinone | $0.0^{c}$ | $2.4^{a}$ | $1.6^{b}$ | $0.0^{c}$ | $0.0^{c}$ |
| 19 | (+)-A-Tocopherol | $0.0^{c}$ | $5.6^{a}$ | $0.0^{c}$ | $1.1^{b}$ | $0.0^{c}$ |
| 20 | Cis-Vaccenic Acid | $0.0^{e}$ | $15.9^{b}$ | $9.0^{c}$ | $30.6^{a}$ | $5.2^{d}$ |
| 21 | 3,5-Di-Tert-Butylcatechol | $0.0^{b}$ | $0.0^{b}$ | $0.5^{a}$ | $0.0^{b}$ | $0.0^{b}$ |
| 22 | (+)-Nerolidol | $0.0^{b}$ | $0.0^{b}$ | $0.8^{a}$ | $0.0^{b}$ | $0.0^{b}$ |
| 23 | Lucenin -2 | $0.0^{c}$ | $0.0^{c}$ | $0.9^{b}$ | 0.0 | $2.2^{a}$ |
| 24 | Cholecalciferol | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ | $5.5^{a}$ |
| 25 | 24,25-Dihydroxyvitamin D3 | $0.0^{c}$ | $0.0^{c}$ | $0.0^{c}$ | $0.9^{b}$ | $5.5^{a}$ |
| 26 | Gentamicin A | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ | $0.3^{a}$ |
| 27 | Actinobolin | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ | $1.1^{a}$ |
| 28 | Asteromycin | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ | $0.0^{b}$ | $0.6^{a}$ |
| | **Total** | **100** | **100** | **100** | **100** | **100** |

**Each value in the table was obtained by calculating the average of the three experiments. The superscript letters indicated statistically significant differences, with P <0.05. MSB: *Moringa stenopetala* in Belbis; MSA: *Moringa stenopetala* in Aswan; MON: *Moringa oleifera* in Nubarya; MOS: *Moringa oleifera* in Shalateen; MPG: *Moringa peregrina* in Giza.**

On the other hand, MPG leaves ethanolic extract contain 16 volatile chemical compounds showed in figure (36) and their structures were showed in figures (37-40). MPG recorded the highest values of L-Methionine, Arachidonic Acid, Ethyl Ester, Lucenin -2, Cholecalciferol, Gentamicin A, Actinobolin and Asteromycin (13.7, 47.7, 2.2, 5.5, 0.3, 1.1 and 0.6 %) respectively.

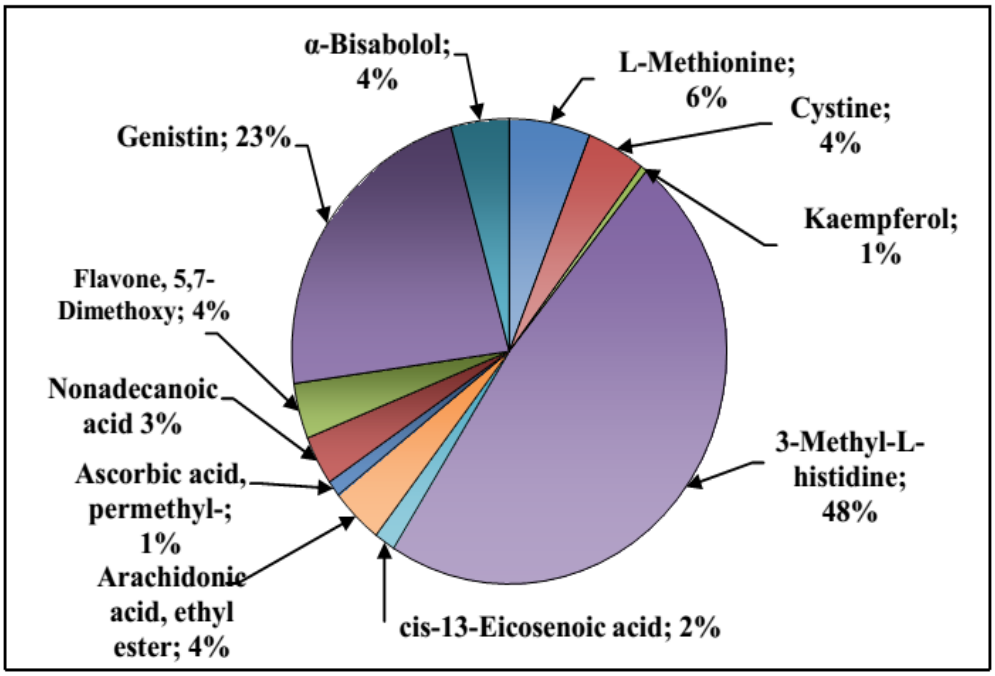



**Figure 32. Chemical compound percentages of functional groups for *Moringa stenopetala* leaves in belbis, determined by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**

**Table 17. Chemical functional groups, chemical symbols and Molecular Weight for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves extract.**

| No. | Functional Group | Compound Name | Chemical Symbol | M.W |
|---|---|---|---|---|
| 1 | AA | L-Methionine | $C_5H_{11}NO_2S$ | 149 |
| 2 | AA | Cysteine | $C_3H_7NO_2S$ | 121 |
| 3 | AA | 3-Methyl-L-histidine | $C_7H_{11}N_3O_2$ | 169 |
| 4 | AA | Glycylglycine | $C_4H_8N_2O_3$ | 132 |
| 5 | Vitamin C | Ascorbic acid, permethyl- | $C_{18}H_{40}O_6Si_4$ | 464 |
| 6 | Vitamin A | (+)-α-Tocopherol | $C_{29}H_{50}O_2$ | 430 |
| 7 | Vitamin $D_3$ | 24,25-Dihydroxyvitamin $D_3$ | $C_{27}H_{44}O_3$ | 416 |
| 8 | FA | Arachidonic acid, ethyl ester | $C_{22}H_{36}O_2$ | 332 |
| 9 | FA | Nonadecanoic acid | $C_{19}H_{38}O_2$ | 298 |
| 10 | FA | cis-13-Eicosenoic acid | $C_{20}H_{38}O_2$ | 310 |
| 11 | FA | cis-Vaccenic acid | $C_{18}H_{34}O_2$ | 282 |
| 12 | FA | (+)-Nerolidol | $C_{15}H_{26}O$ | 222 |
| 13 | FA | Linolenic acid | $C_{18}H_{30}O_2$ | 278 |
| 14 | FA | Octanoic acid | $C_8H_{16}O_2$ | 144 |
| 15 | OA | n-Butylphosphonic acid | $C_4H_{11}O_3P$ | 138 |
| 16 | Terpenoid | Kaempferol | $C_8H_8O_4$ | 168 |
| 17 | Terpenoid | α-Bisabolol | $C_{15}H_{26}O$ | 222 |
| 18 | Phenol | 2,6-Di-tert-butylhydroquinone | $C_{14}H_{21}NO_2$ | 235 |
| 19 | Phenol | 3,5-di-tert-Butylcatechol | $C_{14}H_{22}O_2$ | 222 |
| 20 | Flavonoid | Quercetin 3',4',7-Trimethyl Ether | $C_{18}H_{16}O_7$ | 344 |
| 21 | Flavonoid | Genistin | $C_{21}H_{20}O_{10}$ | 432 |
| 22 | Flavonoid | Flavone, 5,7-Dimethoxy- | $C_{17}H_{14}O_4$ | 282 |
| 23 | Flavonoid | Geranyl iso-valerate | $C_{15}H_{26}O_2$ | 238 |
| 24 | Flavonoid | Lucenin -2 | $C_{27}H_{30}O_{16}$ | 610 |
| 25 | Flavonoid | Cholecalciferol | $C_{27}H_{44}O$ | 384 |
| 26 | Flavonoid | Gentamicin A | $C_{18}H_{36}N_4O_{10}$ | 468 |
| 27 | Flavonoid | Actinobolin | $C_{13}H_{20}N_2O_6$ | 300 |
| 28 | Flavonoid | Asteromycin | $C_{16}H_{25}N_7O_8$ | 443 |

**Chemical compounds detected by gas chromatography mass analysis.**

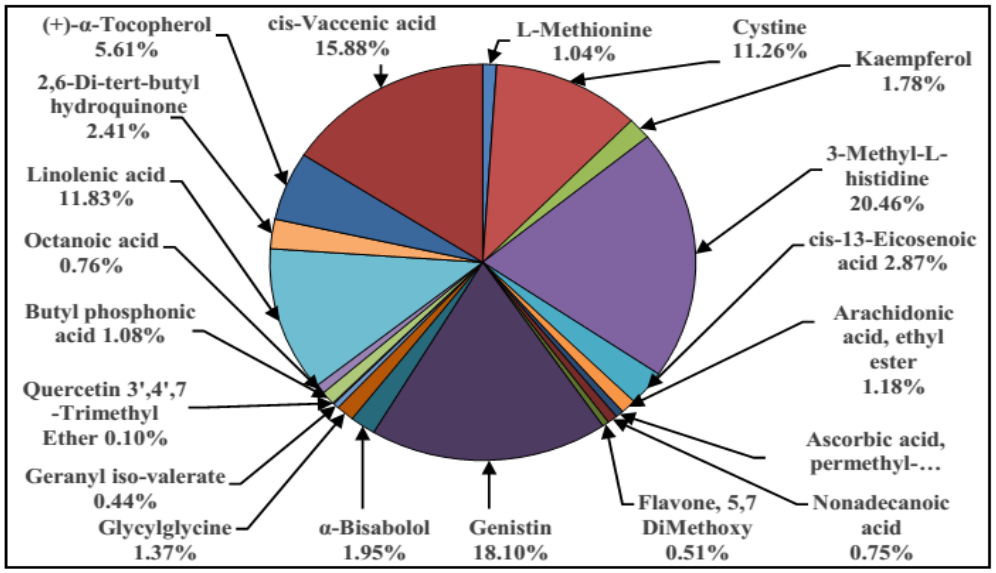



**Figure 33. Chemical compound percentages of functional groups for *Moringa stenopetala* leaves in Aswan, determined by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**

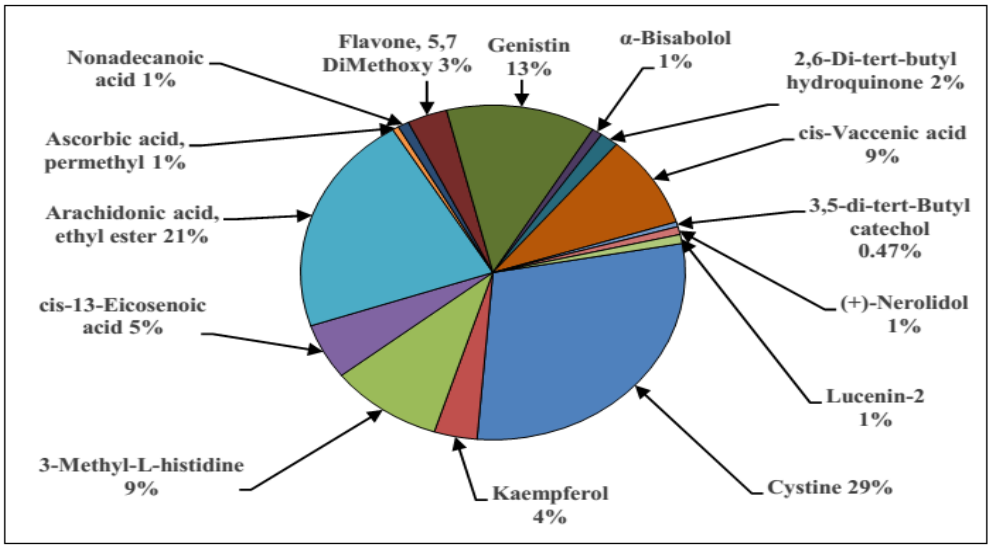



**Figure 34. Chemical compound percentages of functional groups for *Moringa oleifera* leaves in Nubarya, determined by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**

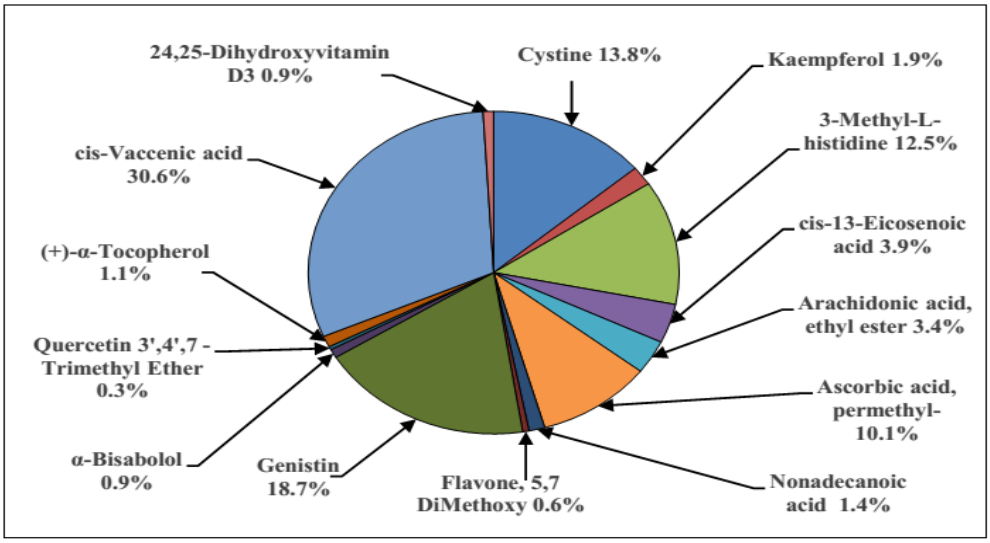



**Figure 35. Chemical compound percentages of functional groups for *Moringa oleifera* leaves in Shalateen, determined by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**

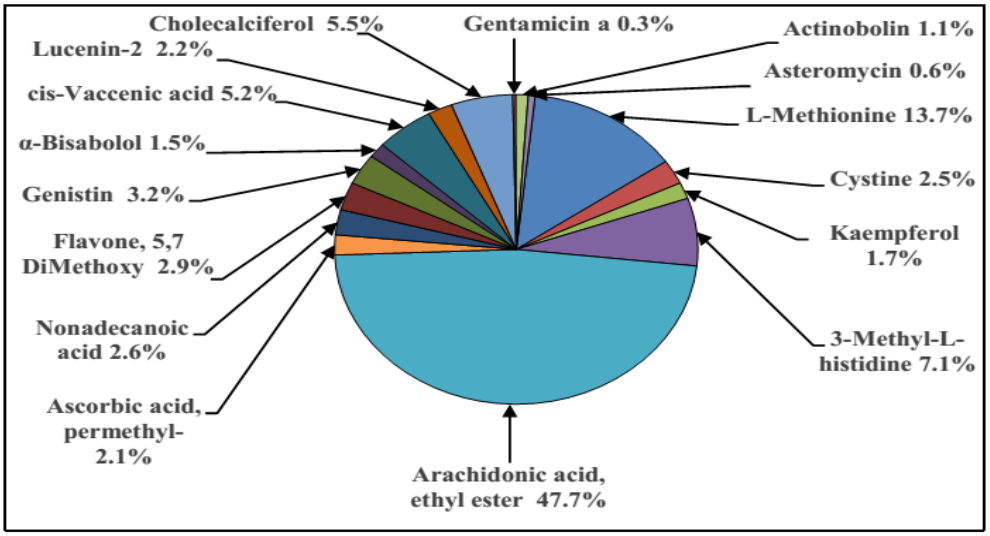



**Figure 36. Chemical compound percentages of functional groups for *Moringa peregrina* leaves in Giza, determined by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**

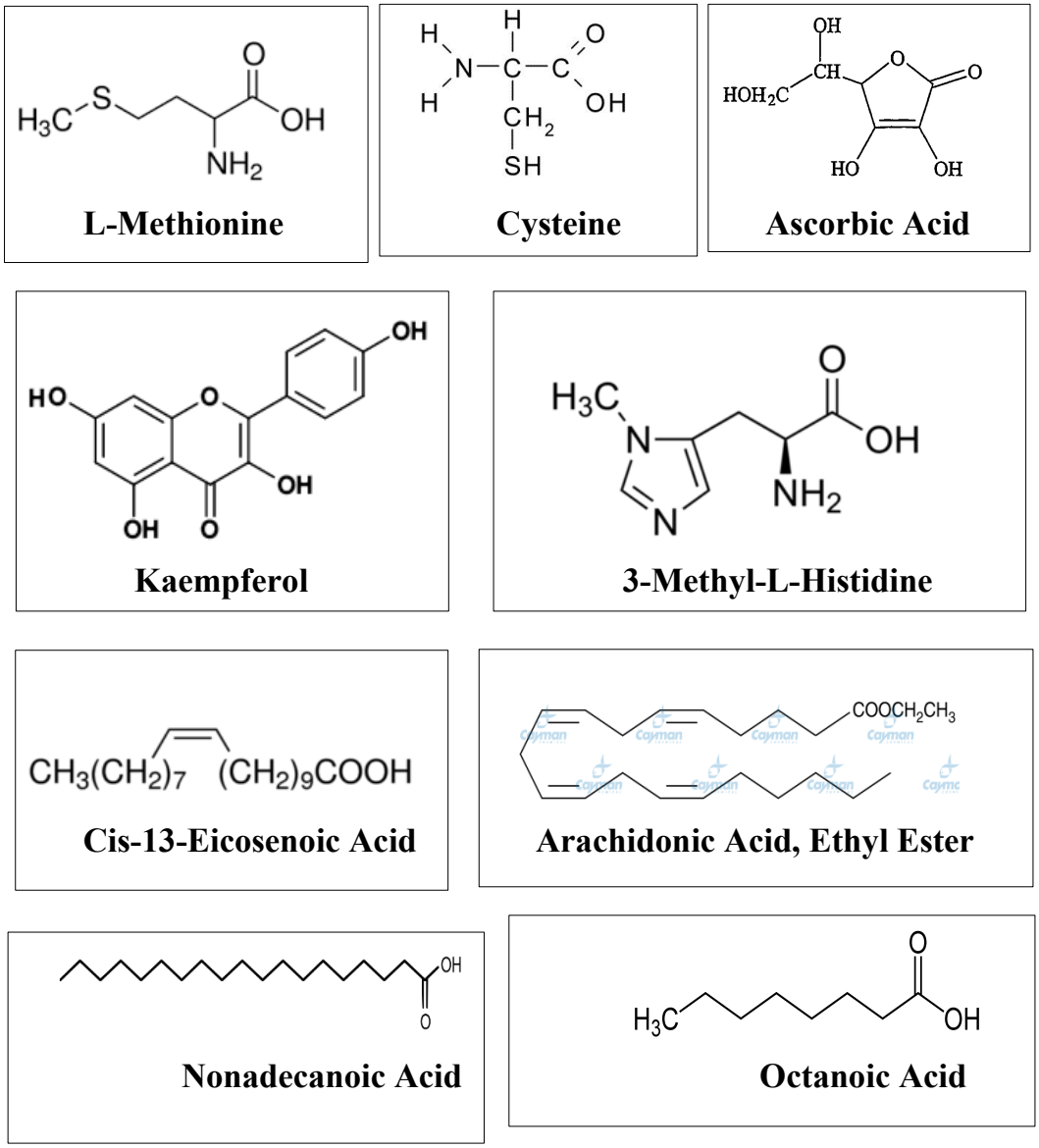



**Figure 37. Chemical composition of functional groups for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**

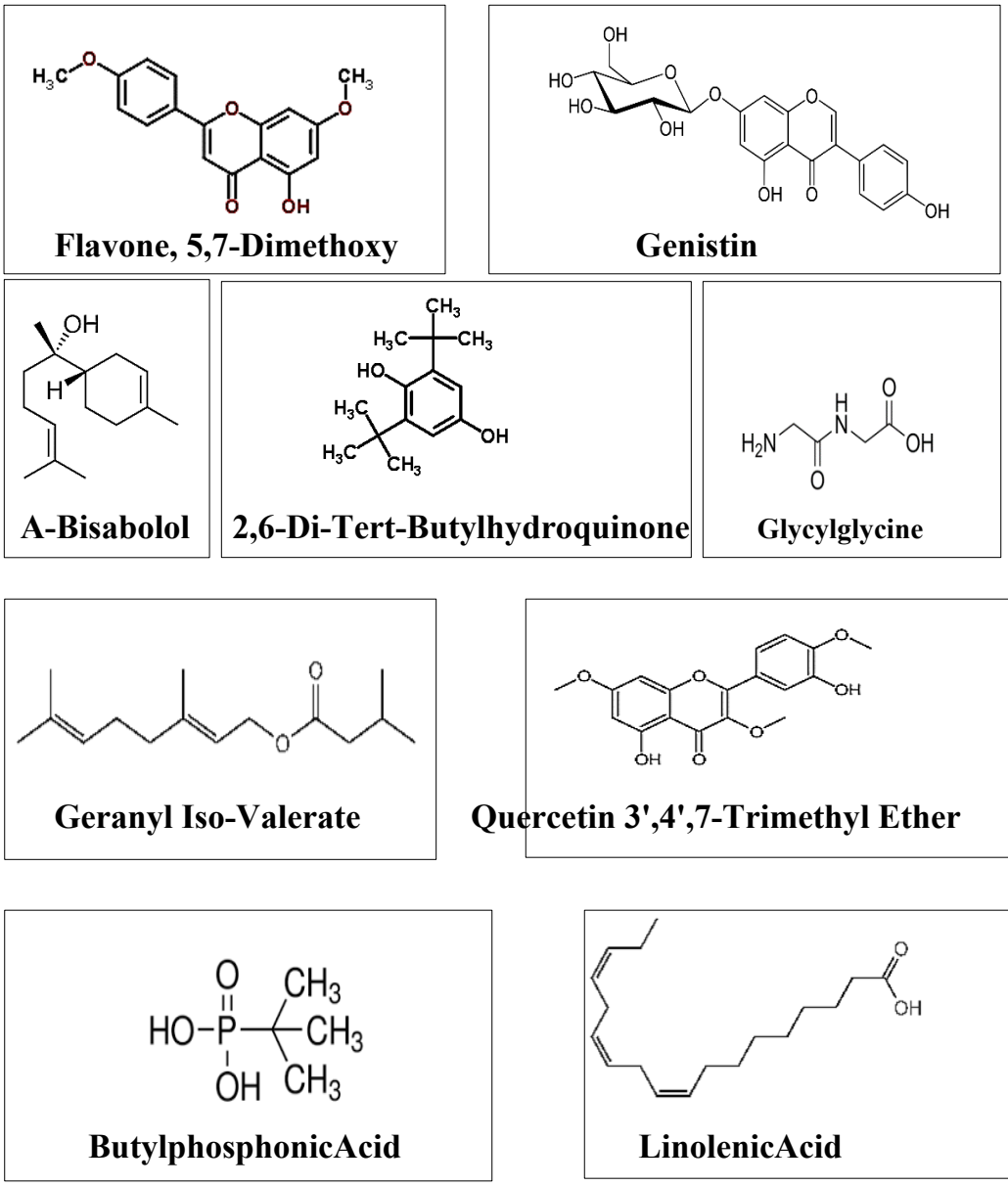
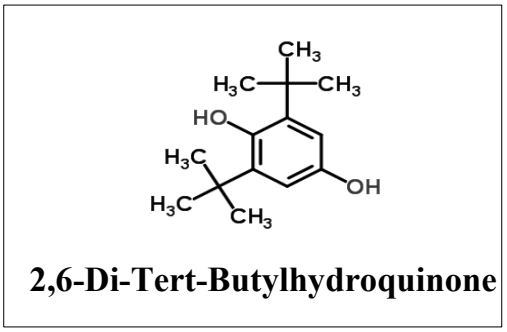
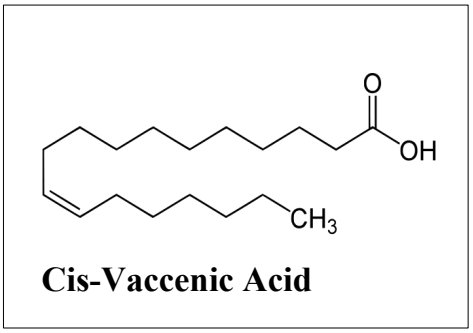
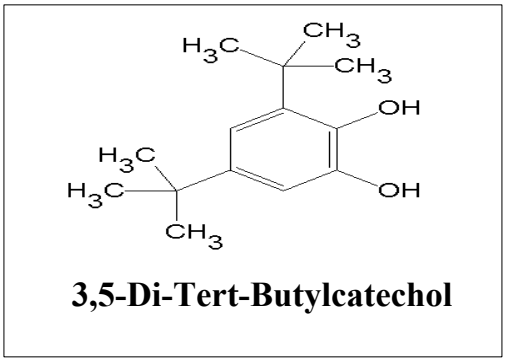
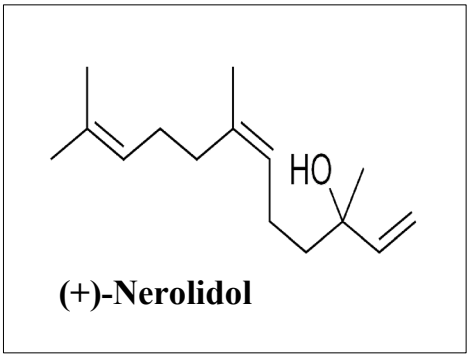


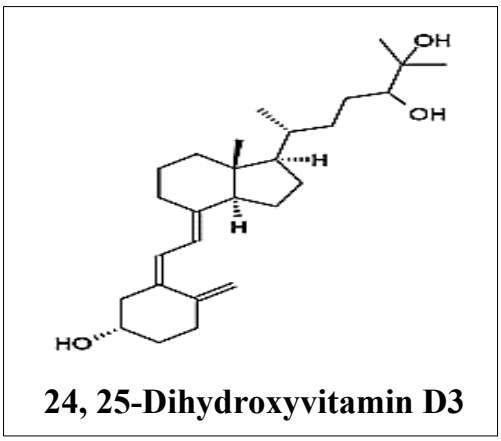

**Figure 38. Chemical composition of functional groups for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**



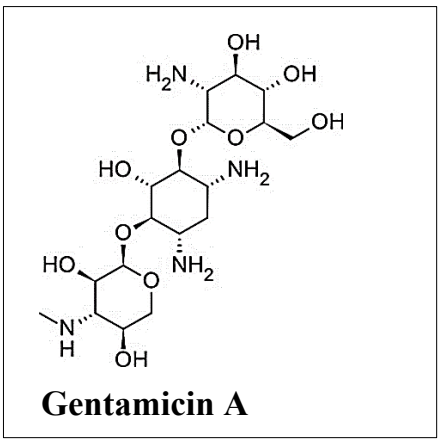




**Figure 39. Chemical composition of functional groups for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**

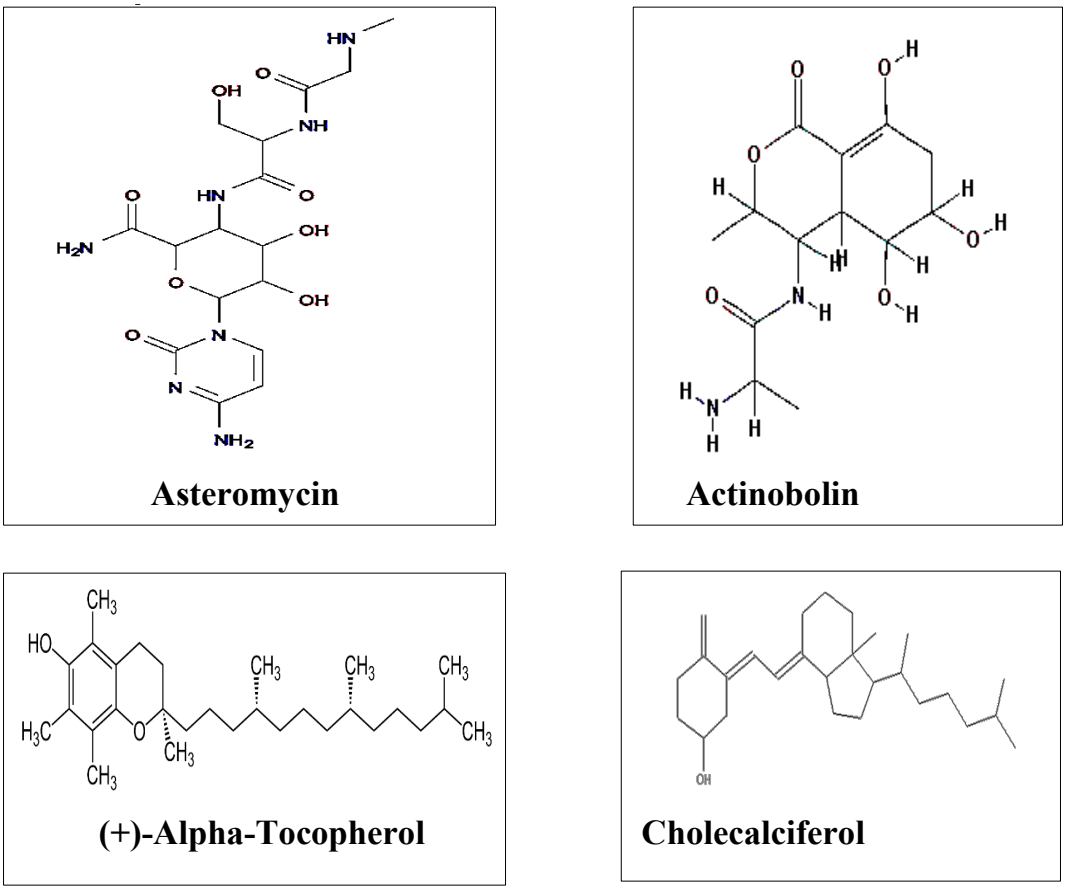



**Figure 40. Chemical composition of functional groups for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* by gas chromatography mass, (NIST and WILEY library by Lehotay, 2002).**

## 4.14. Biochemical parameters of first biological experiment

Acute STZ-induced diabetic albino rat's studies were treated with aqueous and ethanolic extract of *Moringa oleifera* leaves for a period of 30 days for screening and discovering experiment.

The diabetic studies revealed nontoxic nature of the *Moringa oleifera* at a concentration of 600 mg/kg of body weight per day for this period. There

were some morphological changes like weight losses, more drinking water and more urine. There was no lethality or any toxic reactions found at either doses selected till the end of treatment period.

### 4.14.1. Blood glucose levels

The data presented in table (18) showed that; Blood glucose levels of STZ-induced diabetic groups, there was a significant different among induced-STZ treatment groups on fasting blood glucose, that mean good affecting of that dose of induced STZ (65mg/kg b.wt) and its components inhibited the destruction of rat pancreatic islet β-cells, when compared with control group 1.

On the other hand, while glucose level postprandial 2 hrs. was significantly high in diabetic control group, the level of blood glucose was significantly decreased in groups 4, 5 and 7 compared with diabetic group 2 (244.3, 274.7, 249.3 and 420.0 mg/dl) respectively.

With agreement of a significant reduction in plasma glucose concentration levels were observed after administration of STZ and (250, 500 mg/kg b.w) of *Moringa* leaves extract (MLE), (250, 500 mg/kg b.w) of *Moringa* stems extract (MSE) and (250, 500 mg/kg b.w) of *Moringa* pods extract (MPE), in diabetic rats, which recorded (168, 160, 186, 183, 177, 175 mg/dl) respectively, compared with control and diabetic groups (150 and 280 mg/dl) respectively. Trend was MLE > MPE ≈ MSE effective on plasma glucose level, (Sushma *et al.,* 2013).

In addition, there were significant different observed on postprandial 4 hrs. blood glucose levels among groups 4, 5 and 7 compared with diabetic group 2 (278.7, 283.3, 251.0 and 407.3 mg/dl) respectively, the same results were observed on postprandial 6 hrs. blood glucose levels among groups 4 and 7 compared with diabetic group 2 (277.3, 284.7 and 427.3 mg/dl) respectively.

**Table 18. Effects of *Moringa oleifera* extracts on fasting and postprandial blood glucose levels of diabetic rats.**

| Treatment | FBG (mg/dl) | PPBG (mg/dl) | | |
|---|---|---|---|---|
| | | 2hrs | 4hrs | 6hrs |
| **Group1** | $93.7^{f}$±0.87 | $87.7^{f}$±1.15 | $86.0^{f}$±1.45 | $92.7^{f}$±1.86 |
| **Group 2** | $447.3^{a}$±3.76 | $420.0^{a}$±4.41 | $407.3^{a}$±3.48 | $427.3^{a}$±3.79 |
| **Group 3** | $436.3^{b}$±2.91 | $405.7^{b}$±4.33 | $341.7^{c}$±3.61 | $400.0^{b}$±5.04 |
| **Group 4** | $342.7^{d}$±2.08 | $244.3^{e}$±2.65 | $278.7^{d}$±4.16 | $277.3^{e}$±2.40 |
| **Group 5** | $302.0^{e}$±2.03 | $274.7^{d}$±3.76 | $283.3^{d}$±2.33 | $311.3^{d}$±4.10 |
| **Group 6** | $393.0^{c}$±4.62 | $374.0^{c}$±2.65 | $354.3^{b}$±2.34 | $385.3^{c}$±3.48 |
| **Group 7** | $304.7^{e}$±4.36 | $249.3^{e}$±2.33 | $251.0^{e}$±2.32 | $284.7^{e}$±2.91 |
| **LSD(0.05)** | **5.23** | **7.19** | **4.72** | **6.63** |

**Each value in the table was obtained by calculating the average of the three experiments ±S.E. The superscript letters indicated statistically significant differences, with P <0.05.**

### 4.14.2. Effect of *Moringa oleifera* leaves extract on Alanine aminotransferase, Aspartate aminotransferase and creatinine of diabetic rats.

The data presented in table 19 illustrated by figures (41-49) showed that; Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities on STZ-induced diabetic groups. Groups 3, 4, 6 and 7 showed significant decrease compared with diabetic group 2, as (13.5, 12.1, 13.9, 6.2 and 19.4 U/l) respectively.

**Table 19. Effect of *Moringa oleifera* leaves extract on ALT, AST and creatinine of diabetic rats.**

| Treatment | ALT*(U/l) | AST*(U/l) | Creatinine*(mg/dl) |
|---|---|---|---|
| **Group1** | $7.4^{d}\pm0.38$ | $3.7^{d}\pm0.23$ | $0.43^{e}\pm0.10$ |
| **Group 2** | $19.4^{a}\pm1.16$ | $9.0^{a}\pm0.76$ | $1.23^{a}\pm0.06$ |
| **Group 3** | $13.5^{c}\pm0.60$ | $6.3^{bc}\pm0.46$ | $0.65^{d}\pm0.01$ |
| **Group 4** | $12.1^{c}\pm0.72$ | $5.3^{c}\pm0.43$ | $0.65^{d}\pm0.01$ |
| **Group 5** | $17.7^{ab}\pm0.95$ | $4.3^{d}\pm0.41$ | $1.04^{b}\pm0.03$ |
| **Group 6** | $13.9^{c}\pm0.75$ | $7.3^{b}\pm0.43$ | $0.81^{cd}\pm0.03$ |
| **Group 7** | $6.2^{d}\pm0.46$ | $6.0^{bc}\pm0.53$ | $0.90^{bc}\pm0.04$ |
| **LSD (0.05)** | **6.25** | **2.69** | **0.22** |

**Each value in the table was obtained by calculating the average of the three experiments ±S.E. The superscript letters indicated statistically significant differences, with P <0.05. *Normal values: ALT (3-36 U/L); AST (0-35 U/L); Creatinine (0.7-1.4 mg/dl).**

On the other hand group 7 showed a significant decrease in ALT activity in a similar result with control group 1 (6.2 and 7.4 U/l) respectively, while group 5 showed a significant increase in ALT activity comparing result with diabetic control group 2 (17.7and 19.4U/l) respectively.

This result is of special interest which is lower than with previous studies of ALT range (35-48.83 U/l), (Awodele *et al.,* 2012). Also significant decrease on (AST) activity were observed for groups (3, 4, 5, 6 and 7) compared with diabetic group 2 recorded (6.3, 5.3, 4.3, 7.3, 6.0 and 9.0 U/l) respectively.

On the other hand there were no significantly different for AST activity on groups 5 and 1 recorded 4.3 and 3.7 U/l, respectively, This result is of special interest which is lower than with previous studies of AST range 31.47-55.6 U/l, (Awodele *et al.,* 2012).

Creatinine levels recorded significant decrease for groups 3, 4, 5, 6 and 7 compared with diabetic group 2 (0.65, 0.65, 1.04, 0.81, 0.90 and 1.23 mg/dl) respectively, This result is of special interest which is in agreement with previous studies of creatinine range (1.26-2.27 mg/dl), (Awodele *et al.,* 2012).

### 4.14.3. Effect of *Moringa oleifera* leaves extract on triglycerides, total cholesterol, HDL and LDL of diabetic rats.

The data presented in table 20 showed that; triglyceride level was evaluated and there was significant decrease for groups 3, 4, 5 and 6

compared with diabetic group 2 (242.7, 154.0, 161.2, 164.7 and 271.3 mg/dl) respectively.

On the other hand there were no significant different for groups 3 and 7 in a similar result compared with diabetic group 2 (242.7, 256.3 and 271.3 mg/dl) respectively.

Despite the presence of a significant decrease in the results of groups 4, 5, and 6, but there were no significant differences between them recorded (154.0, 161.2 and 164.7 mg/dl) respectively.

Total Cholesterol level was recorded a significant decrease for groups 3, 4, 5, 6 and 7 compared with diabetic group 2 recorded (154.3, 145.7, 160.9, 140.5, 167.7 and 259.8 mg/dl) respectively. Although, there was a significant decrease in the results of groups 3 and 5 but there were no significant differences between them. Significant increase on HDL level was detected for groups 3, 4, 5 and 7 compared with control group 2 (65.8, 76.7, 59.3, 47.2, 73.3 and 42.1 mg/dl), respectively.

On the other hand there were no significant different for groups 4 and 7 but there was a significant decrease on HDL level compared with control group 1 recorded 76.7, 73.3 and 90.4 mg/dl, respectively.

Group 6 recorded a significant decrease of HDL level, in a similar result of diabetic control group 2 (47.2 and 42.1 mg/dl), respectively. Significant decrease on LDL level was recorded for groups 3, 4, 5, 6 and 7 compared with control group 2 recorded 40.0, 38.2, 69.4, 60.4, 43.1 and 163.5 mg/dl, respectively.

**Table 20. Effect of *Moringa oleifera* leaves extract on triglycerides, total cholesterol, HDL and LDL of diabetic rats.**

| Treatment | Triglycerides* (mg/dl) | Total* Cholesterol (mg/dl) | HDL* (mg/dl) | LDL* (mg/dl) |
|---|---|---|---|---|
| **Group1** | 97.3 $^{d}$±0.39 | 226.7$^{b}$±0.87 | 90.4$^{a}$±1.15 | 115.8$^{b}$±1.01 |
| **Group 2** | 271.3$^{a}$±0.39 | 259.8 $^{a}$±1.63 | 42.1$^{e}$±0.88 | 163.5$^{a}$±1.36 |
| **Group 3** | 242.7$^{b}$±0.49 | 154.3$^{d}$±2.03 | 65.8$^{c}$±1.5 | 40.0$^{d}$±1.04 |
| **Group 4** | 154.0$^{c}$±0.88 | 145.7$^{e}$±2.60 | 76.7$^{b}$±1.5 | 38.2$^{d}$±0.50 |
| **Group 5** | 161.2$^{c}$±0.87 | 160.9$^{cd}$±0.88 | 59.31$^{d}$±0.66 | 69.4$^{c}$±1.13 |
| **Group 6** | 164.7$^{c}$±1.86 | 140.5$^{e}$±0.88 | 47.2$^{e}$±4.06 | 60.4$^{c}$±0.72 |
| **Group 7** | 256.3$^{ab}$±0.88 | 167.7$^{c}$±1.15 | 73.3$^{b}$±2.47 | 43.1$^{d}$±0.81 |
| **LSD(0.05)** | **4.37** | **5.37** | **3.25** | **6.20** |

**Each value in the table was obtained by calculating the average of the three experiments ±S.E. The superscript letters indicated statistically significant differences, with P <0.05. *Normal values: Triglycerides (<195 mg/dl); Total Cholesterol (<200 mg/dl); HDL (<40 mg/dl); LDL (<77.3 mg/dl)**

On the other hand there were no significant different between groups 5 and 6, also groups 3, 4 and 7 were recorded similar result and there was no significant different among them too.

## 4.15. Histopathological examination of first experiment

The main histopathological alterations in the present study were observed in order mainly in the liver, kidneys, and pancreas tissues of STZ-

induced diabetic rats in different groups treated with ethanolic and aqueous extract of *Moringa oleifera* leaves powder.

From the histopathological examination of the tissues; liver, kidneys and pancreas of STZ-induced diabetic rats with three different treated groups 3, 4 and 5 with ethanolic extract of *Moringa oleifera* leaves powder (300, 450 and 600 mg/kg body weight) respectively and groups 6 and 7 treated with aqueous extract of *Moringa oleifera* leaves powder (190 and 225 mg/kg body weight) respectively compared to both normal control group 1 and diabetic control group 2.

### 4.15.1. Histopathological of liver structures

As shown in figures (41 - 47), sections in the liver tissues from untreated rats normal group 1 figure 41 showed that there was normal appearing in hepatic lobular architectures, with normal vasculature, and portal areas. Microscopically examination of liver sections from normal group 1 revealed a normal histological structure of hepatic lobule which consists of central vein and concentrically arranged hepatocytes.

Meanwhile, examined sections from diabetic control group 2 figure 42 hepatic tissue of rat, showing degenerative changes of hepatocytes and granulation of the cytoplasm showing granulation and minute vacuolation of the cytoplasm with proliferation of Van Kupffer phagocytic cells, Kupffer cells (Phagocytic cells of the mononuclear phagocyte series found on the luminal surface of the hepatic sinusoids.) activation, centrilobular hepatic

lipidosis, cytomegally of hepatocytes with foamy cytoplasm and pyknotic nuclei.

Meanwhile, examined sections from group 3 treated with ethanolic extract of *Moringa oleifera* leaves powder (300 mg/kg body weight) illustrated in figure 43 the hepatic tissue of diabetic rat, showing Kupffer cells activation and granularity of the cytoplasm of hepatocytes

However, liver sections of rats from group 4 treated with ethanolic extract of *Moringa oleifera* leaves powder (450 mg/kg body weight) showed in figure 44 showing degenerative changes of the hepatic cells and activation of Van Kupffer cells, dilatation of portal area with proliferation of newly formed bile ducts and different forms of nacrobiotic changes of hepatocytes, revealed a marked improvement in the histopathological picture. Most examined sections revealed Kupffer cells activation and granularity of the cytoplasm of hepatocytes.

While group 5, treated with ethanolic extract of *Moringa oleifera* leaves powder (600 mg/kg body weight) showed in figure 45 showed vacuolization's of the cytoplasm of hepatocytes with slightly improving of hepatocytes tissue compared with diabetic control group 2. On the other hand the diabetic rat group 6 treated with aqueous extract of *Moringa oleifera* leaves powder (190 mg/kg body weight) respectively, figure 46 showing focal area of hemorrhage and necrosis of hepatic tissues and necrobiotic changes of hepatic cells

Meanwhile, examined sections from diabetic group 7 treated with aqueous extract of *Moringa oleifera* leaves powder (225 mg/kg body weight), figure 47 showed necrobiotic changes of hepatocytes, proliferation of newly formed bile canaliculi at the portal area and different forms of necrobiotic changes of hepatocytes proliferation at the same time did not show any improving of liver tissues, compared with normal group 1.

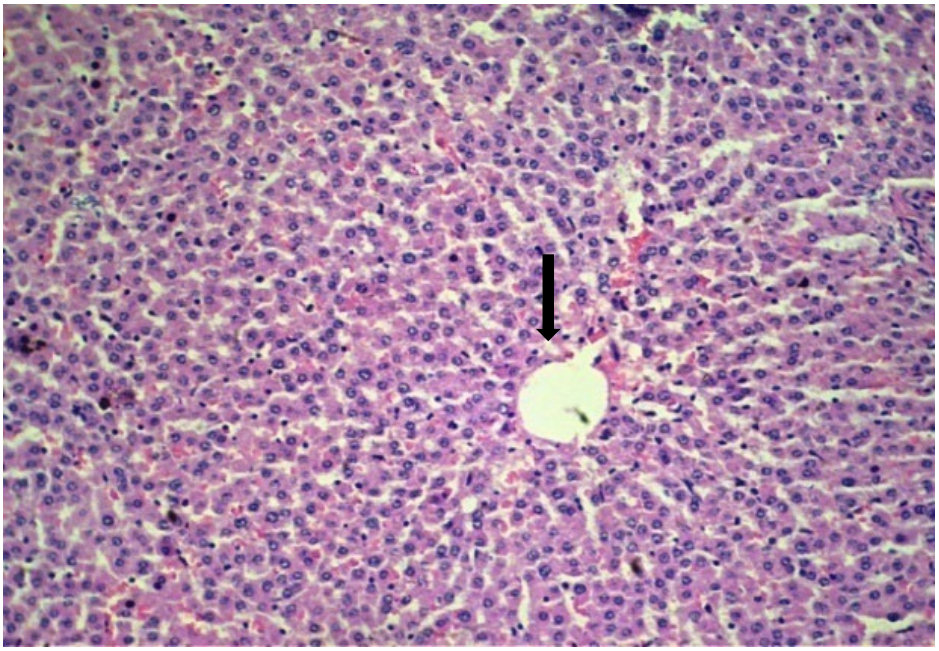


**Fig. 41. Liver of rat from group 1 showing the normal histological structure of hepatic lobule**

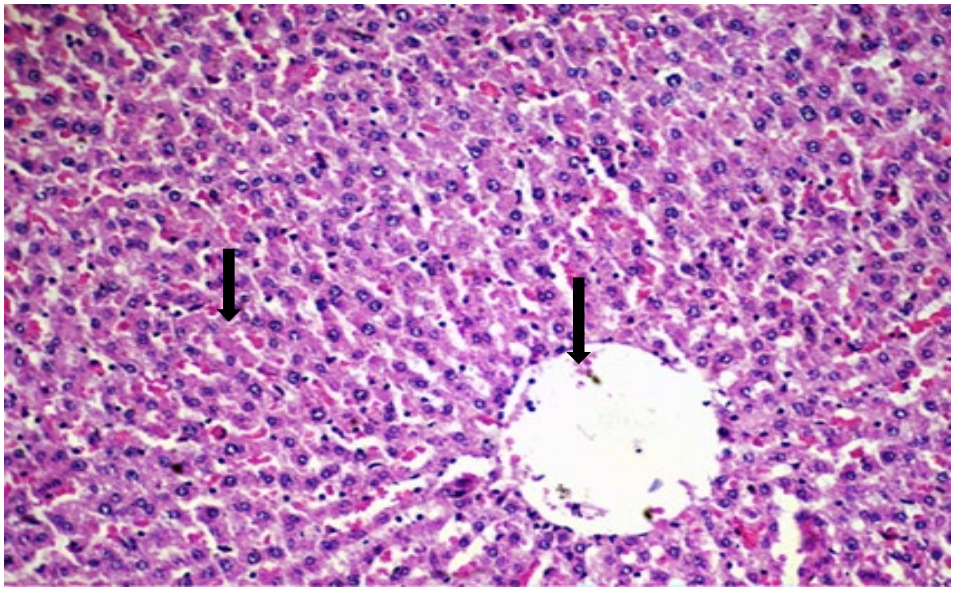


**Fig. 42. Group 2 (diabetic group) showing hepatic tissue of rat, Showing degenerative changes of hepatocytes and granulation of the cytoplasm showing granulation and minute vacuolation of the cytoplasm with proliferation of Van Kupffer phagocytic cells (H &E, X100)**

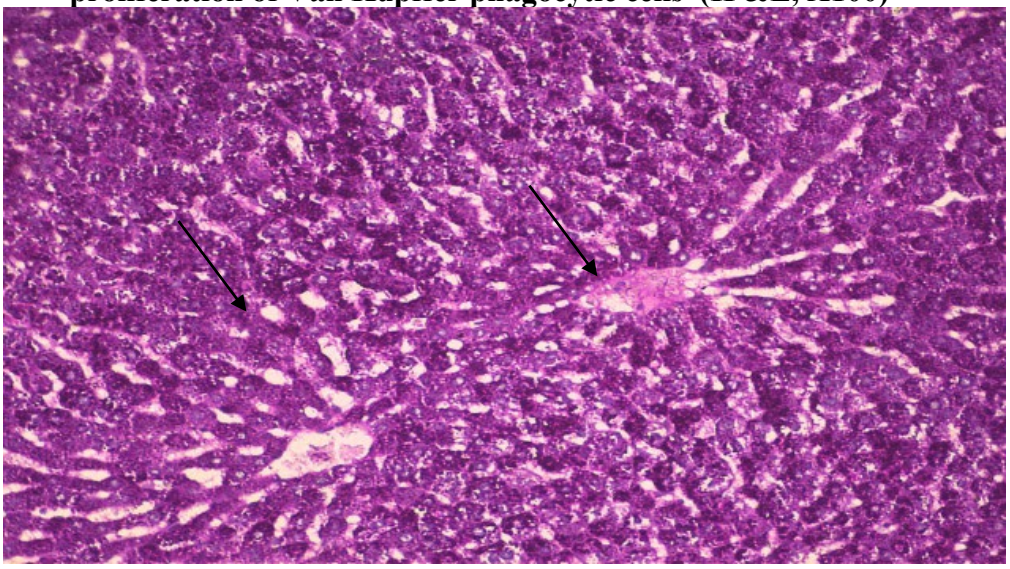


**Fig. 43. Group 3 showing Kupffer cells activation and granularity of the cytoplasm of hepatocytes**

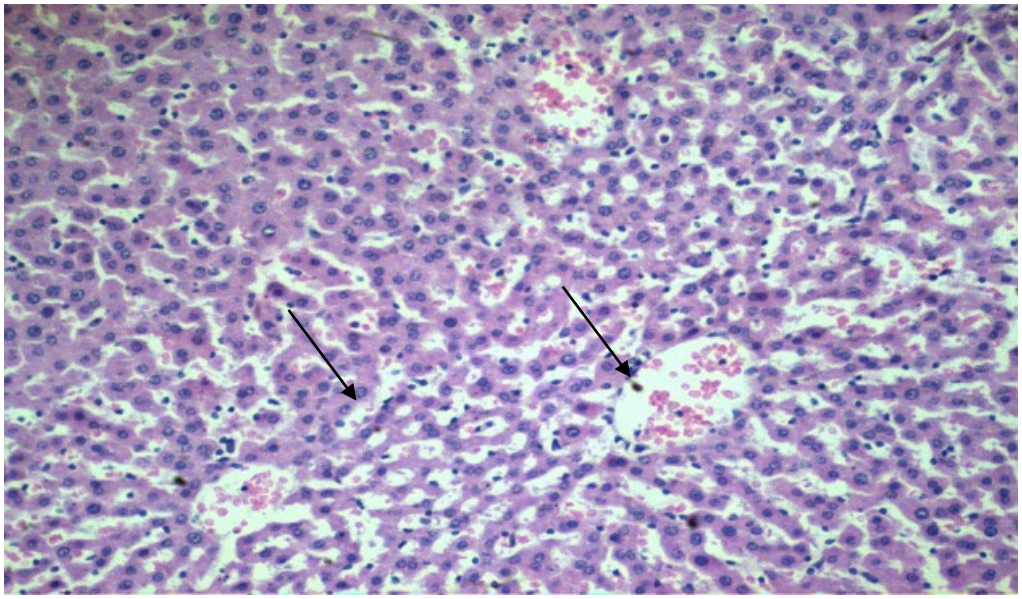


**Fig. 44. Group 4 showing degenerative changes of the hepatic cells and activation of Van Kupffer cells, dilatation of portal area with proliferation of newly formed bile ducts and different forms of nacrobiotic changes of hepatocytes**

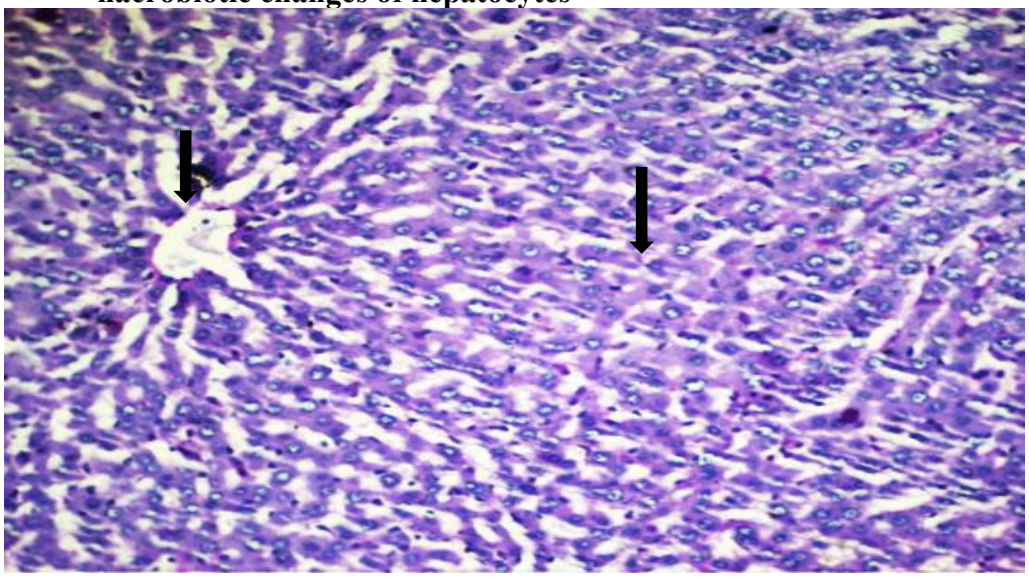


**Fig. 45. Group 5 showing vacuolization's of the cytoplasm of hepatocytes**

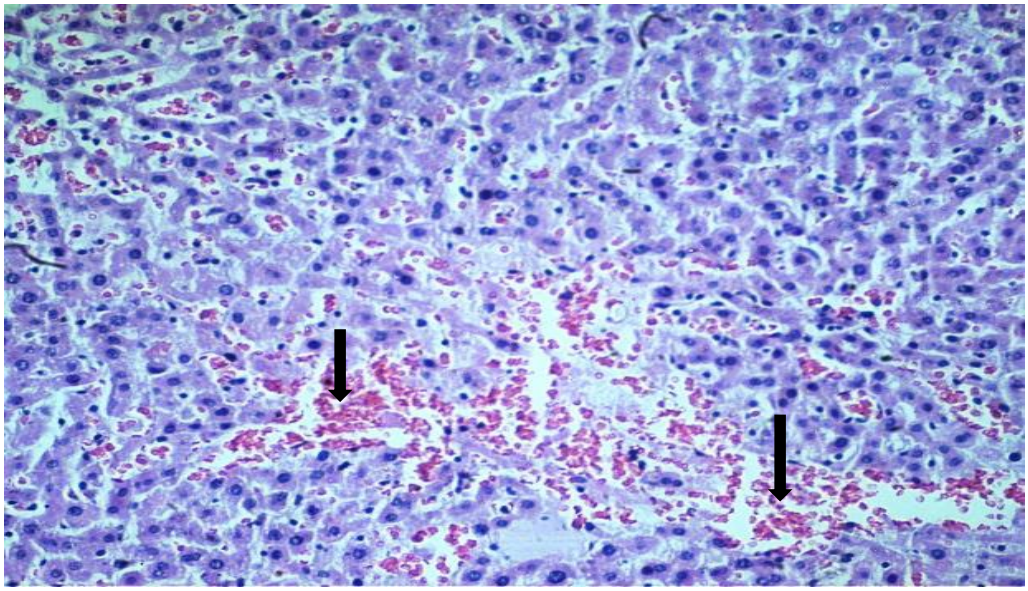


**Fig. 46. Group 6 showing focal area of hemorrhage and necrosis of hepatic tissues and necrobiotic changes of hepatic cells**

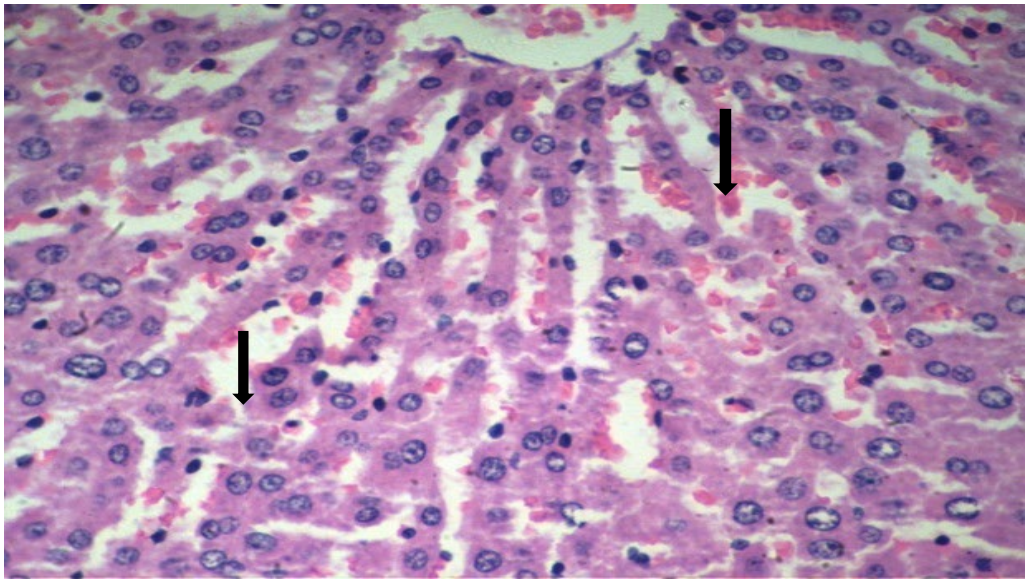


**Fig. 47. Group 7 showing necrobiotic changes of hepatocytes, proliferation of newly formed bile canaliculi at the portal area and different forms of necrobiotic changes of hepatocytes (H & E X 400).**

### 4.15.2. Histopathological of kidneys structures

As shown in figures (48 - 54), sections in the renal tissues from untreated rats normal group (1) Fig. (48) showed the histological of control group 1, showing normal structure of renal tissue as a control.

Meanwhile, examined sections from diabetic control group (2) Fig. (49) renal tissue of rat, showed degenerative changes of renal glomeruli and degenerative changes of epithelial cell of renal tubules.

On the other hand, Renal tissue of group (4) Fig. (51), showed advanced degeneration of renal tubules and damage of most bowman capsules, atrophy and disintegration of the glomerular tuft.

However, kidney sections of rats from group (5) illustrated showed Fig. (52). Renal tissue of group 5, showing degeneration of epithelial cells of renal tubules and infiltration of hyaline material in their lumen and disintegration and atrophy of the glomerular tuft.

Meanwhile, examined sections from diabetic control groups (6 and 7) figures (53 and 54) respectively of renal tissue, showed advanced degeneration of epithelial cells of renal tubules and diffused hyaline cast in their lumen as well as degeneration and necrosis of the endothelial cells of glomerular tuft.

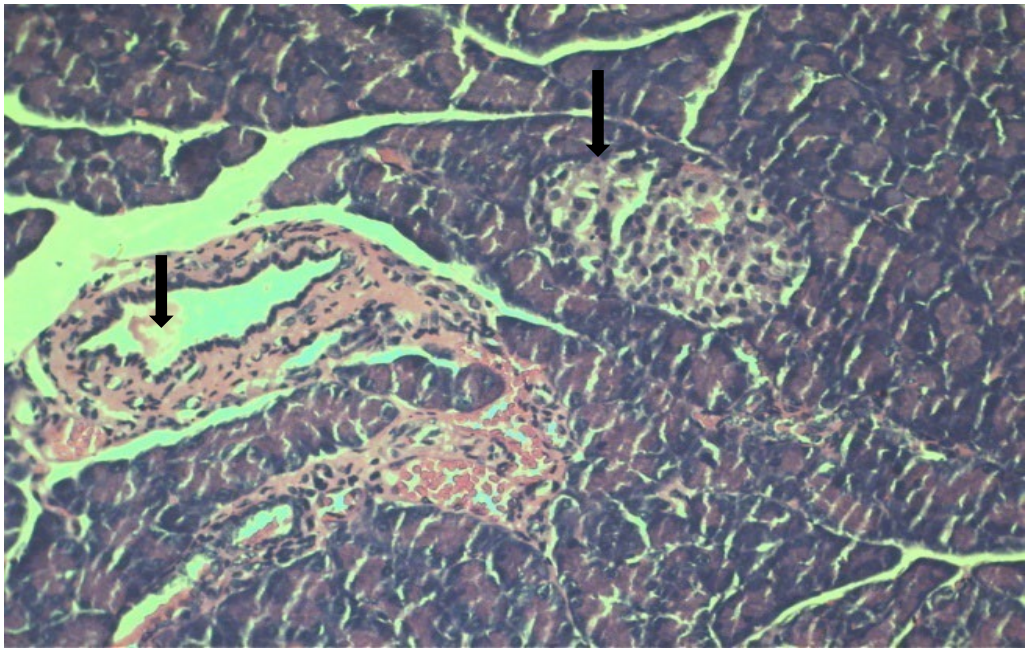


**Fig. 48. The histological of control group 1, showing structure of renal tissue as a control.**

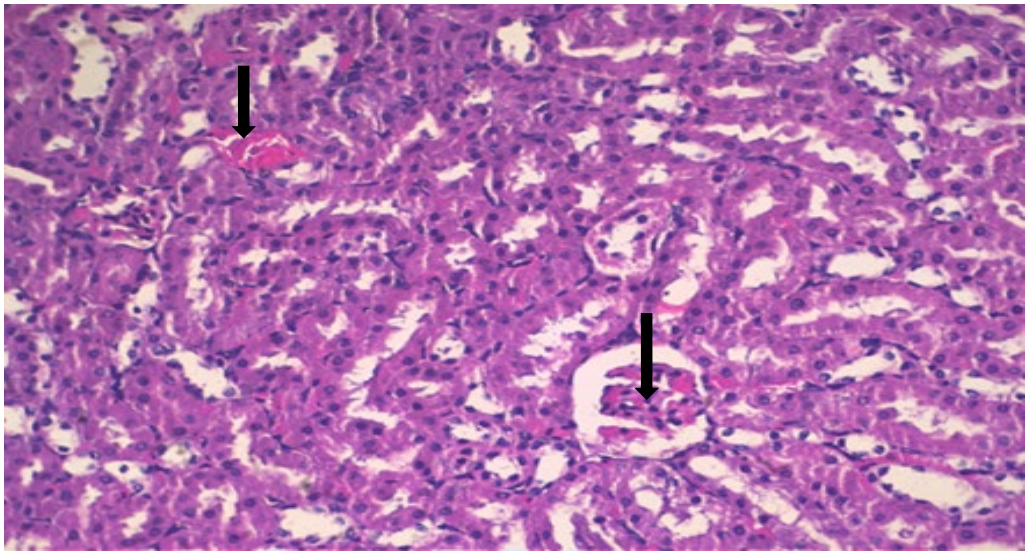


**Fig. 49. Renal tissue of group 2, showing degenerative changes of renal glomeruli and degenerative changes of epithelial cell of renal tubules (H &E, X100).**

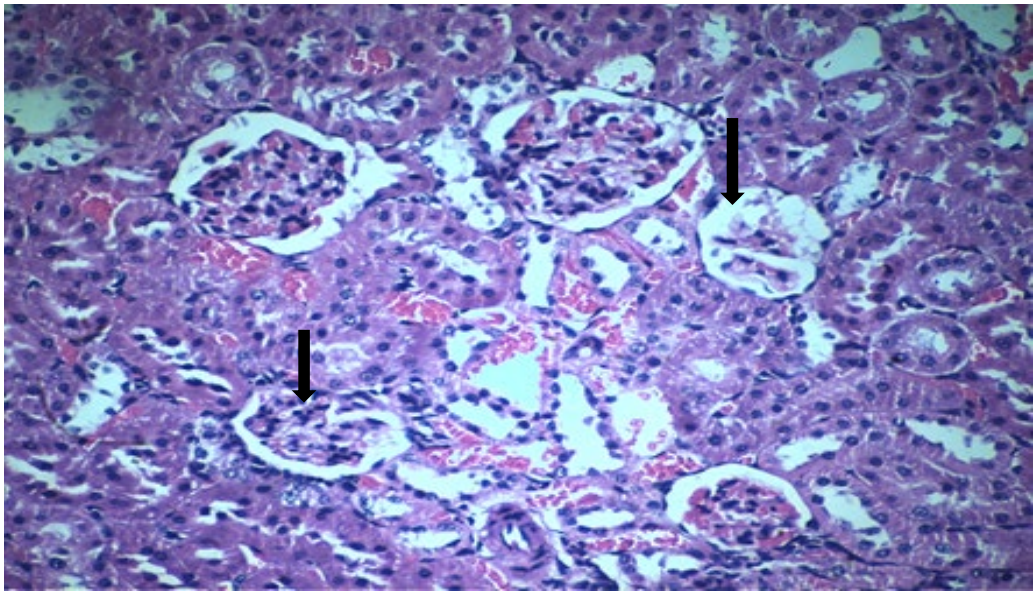


**Fig. 50. Renal tissue of group 4, showing advanced degeneration of renal tubules and damage of most bowman capsules and atrophy and disintegration of the glomerular tuft (H&E, 200).**

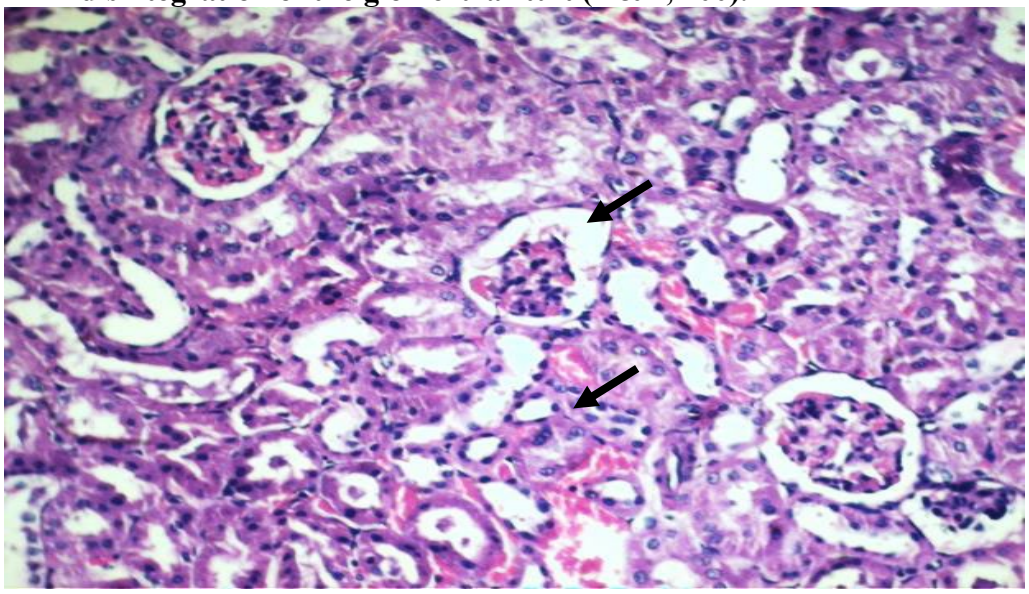


**Fig. 51. Renal tissue of group 5, showing degeneration of epithelial cells of renal tubules and infiltration of hyaline material in their lumen and disintegration and atrophy of the glomerular tuft (H&E, 100).**

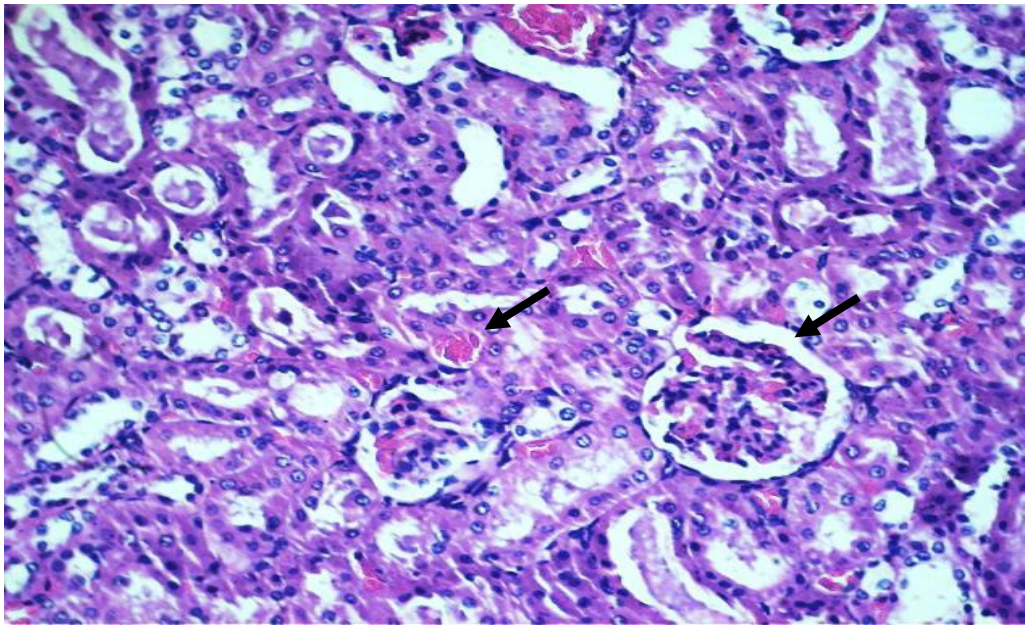


**Fig. 52. Renal tissue of group 5, showing degeneration of epithelial cells of renal tubules and diffused hyaline material in their lumen also, hyaline degeneration in most of glomerular tuft of bowman capsules (H&E, 100).**

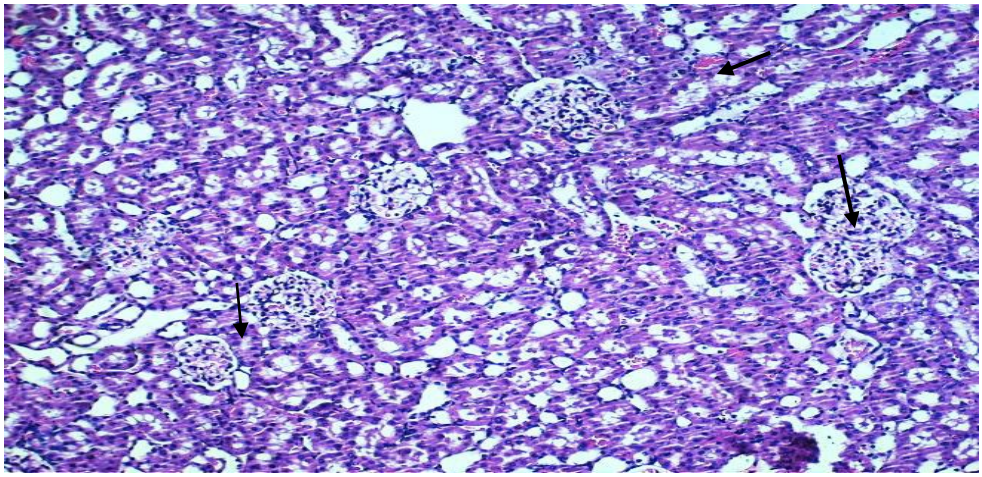


**Fig. 53. Renal tissue of rat group 6, showing advanced degeneration of epithelial cells of renal tubules and diffused hyaline cast in their lumen as well as degeneration and necrosis of the endothelial cells of glomerular tuft ( H&E,100).**

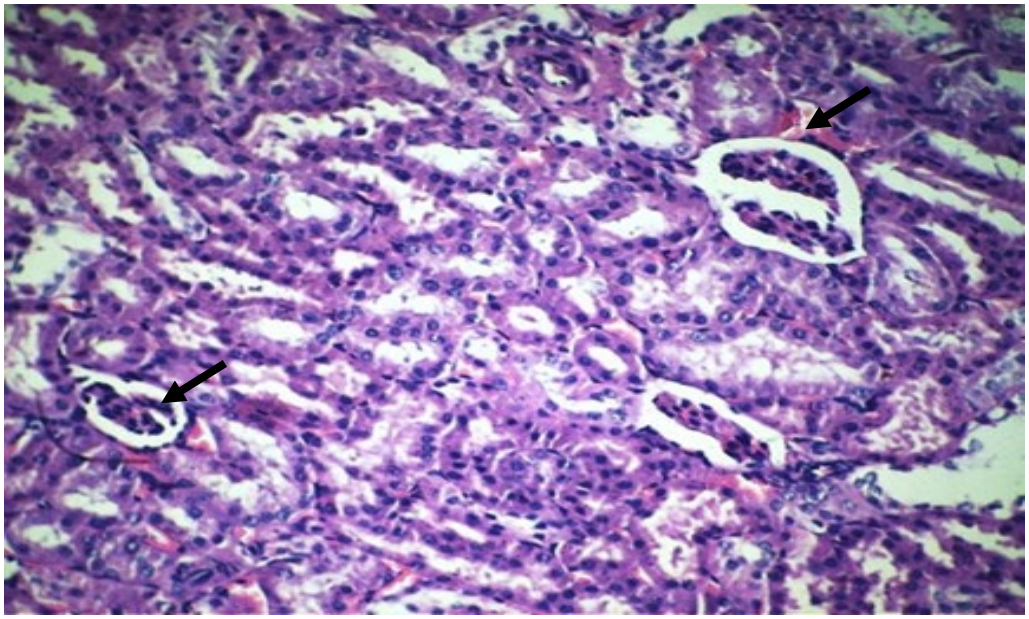


**Fig. 54. Renal tissue of rat group 7, showing advanced degeneration of epithelial cells of renal tubules and diffused hyaline cast in their lumen as well as degeneration, atrophy and collapsed of the endothelial cells of glomerular tuft ( H&E,100).**

### 4.15.3. Histopathological of pancreas structures

As shown in figures (55 - 61), sections in the pancreatic tissues from untreated rats normal group (1) Fig. (55) showed normal pancreatic tissues and atrophy islets of Langerhans.

Meanwhile, examined sections from diabetic control group (2) Fig. (56) pancreatic tissue of diabetic rat, showing marked degeneration and atrophy Islets of Langerhans if compared to the first group.

On the other hand, tissue section pancreas of group (4) figures (57 and 58) showing, very few foci of islets of Langerhans, the cells showed

degenerative changes, increase activating of the exocrine acini portions, with pyknoticnuclei of secretory cells.

On the other hand, tissue section pancreas of groups (5 and 6) Fig. (59 and 60) respectively, showing degenerative an atrophy of islets of Langerhans cells.

Meanwhile, examined tissue section pancreas of group (7) Figures (61 and 62) pancreatic tissue of diabetic rat, showed absence and atrophy of islets of Langerhans cells, and by use high magnification of tissue pancreas section of group (7), showed increase activating of the exocrine acini portions.

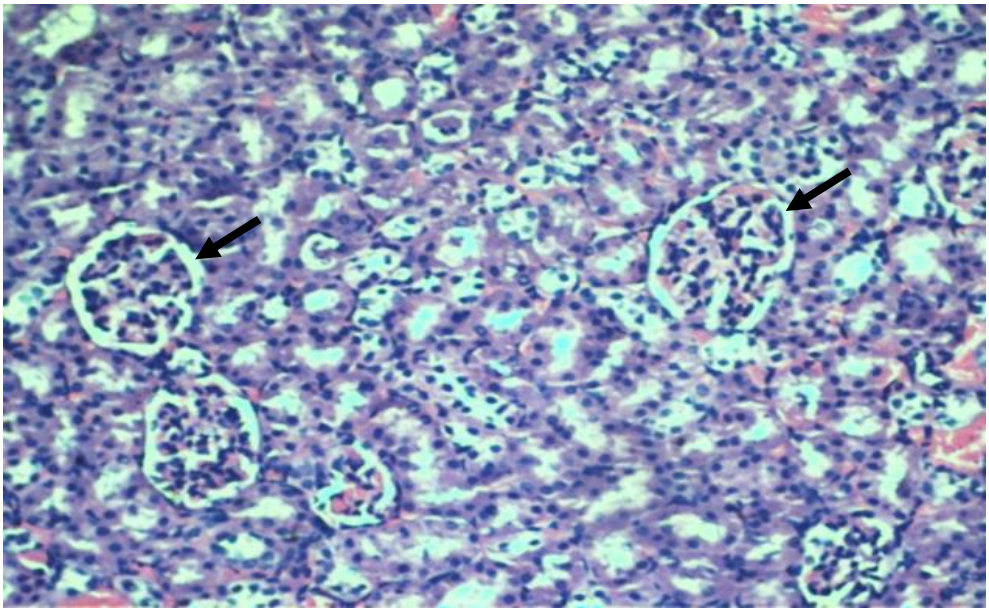


**Fig. 55. Tissue pancreas section of group 1, showing normal tissue degeneration and atrophy Islets of Langerhans (H &E, X100).**

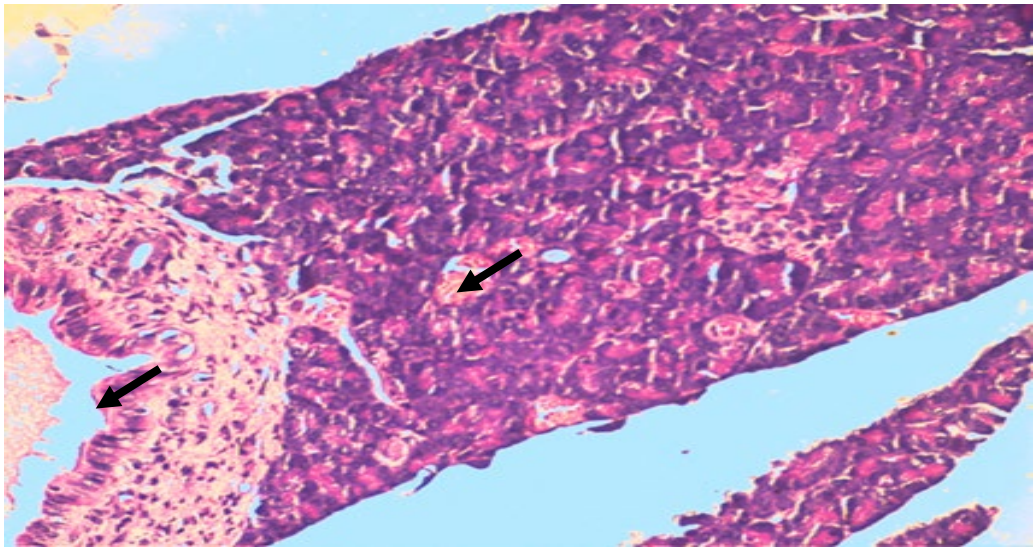


**Fig. 56. Tissue section pancreas of group 2, showing marked degeneration and atrophy Islets of Langerhans if compared to the first group (H &E, X100).**

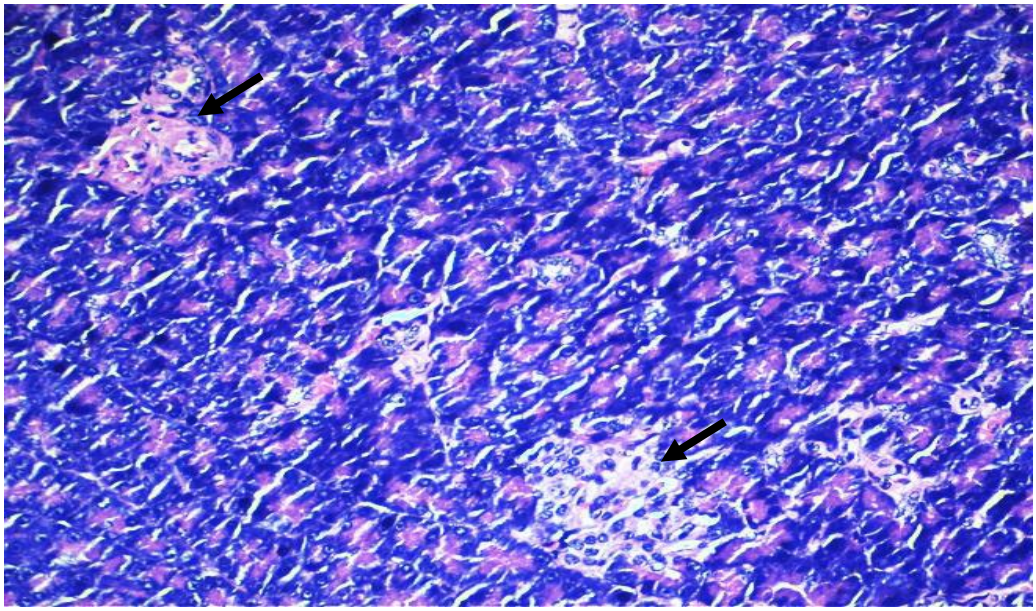


**Fig. 57. Tissue section pancreas of group 4 showing, very few foci of islets of Langerhans, the cells showing degenerative changes. H&E.X100).**

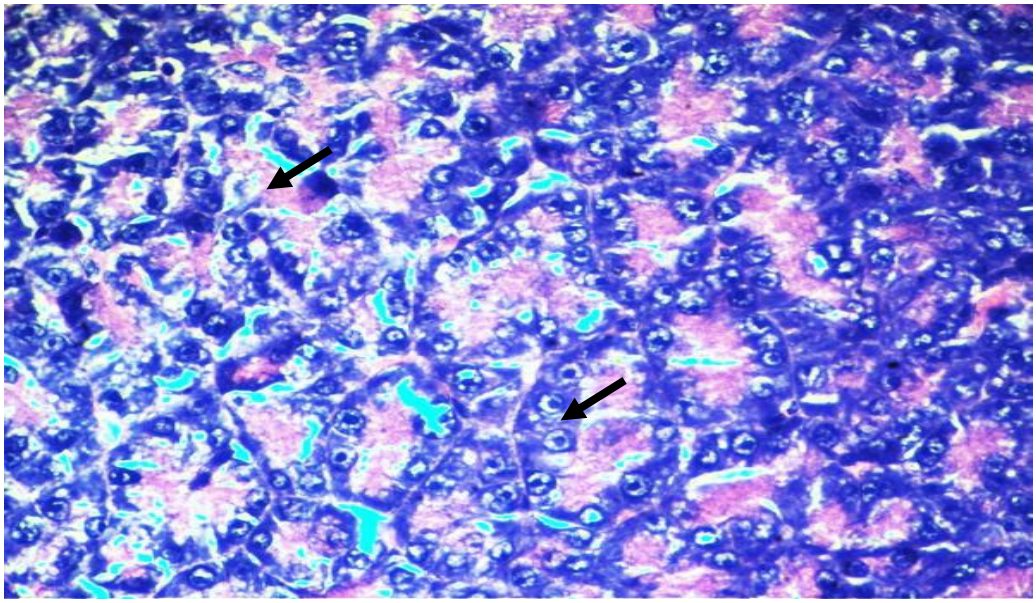


**Fig. 58. High magnification of Tissue section pancreas of group 4, showing increase activating of the exocrine acini portions, with pyknoticnuclei of secretory ells.( H &E, 200).**

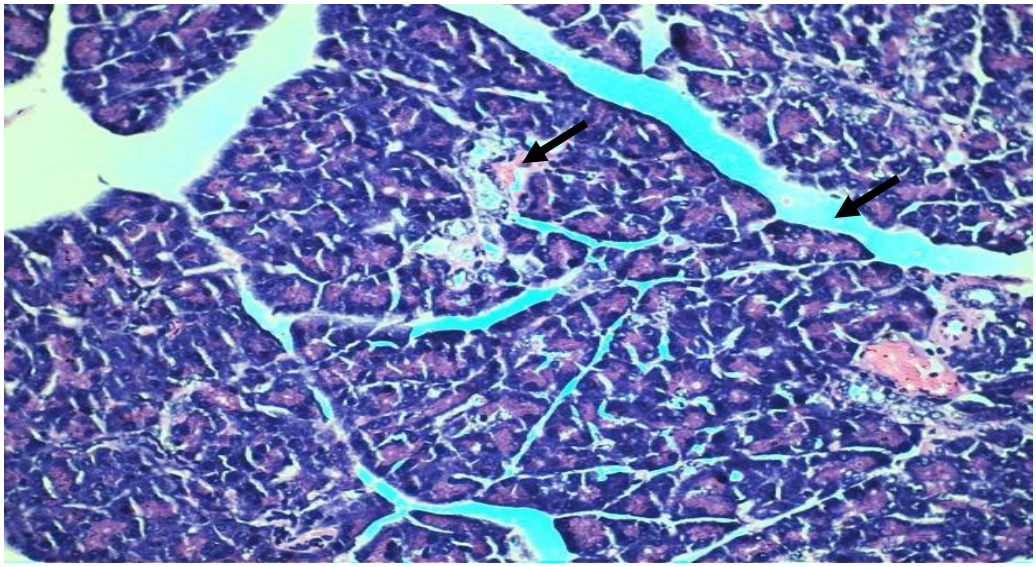


**Fig. 59. Tissue section pancreas of group 5, showing degenerative changes of islets of Langerhans cells (H&E, X100).**

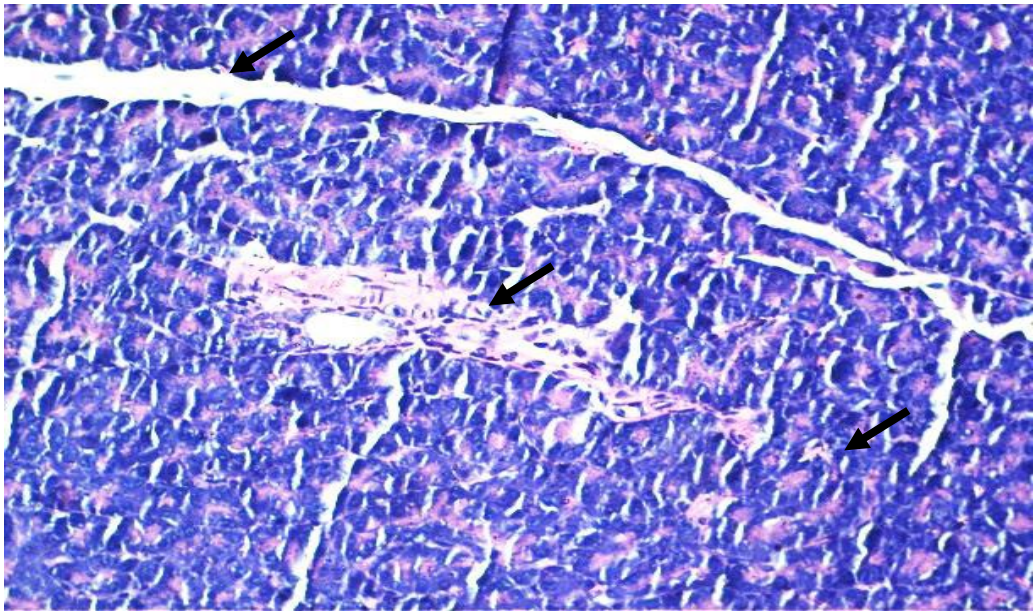


**Fig. 60. Tissue section pancreas of group 6, showing degenerative an atrophy of islets of Langerhans cells (H&E, X100).**

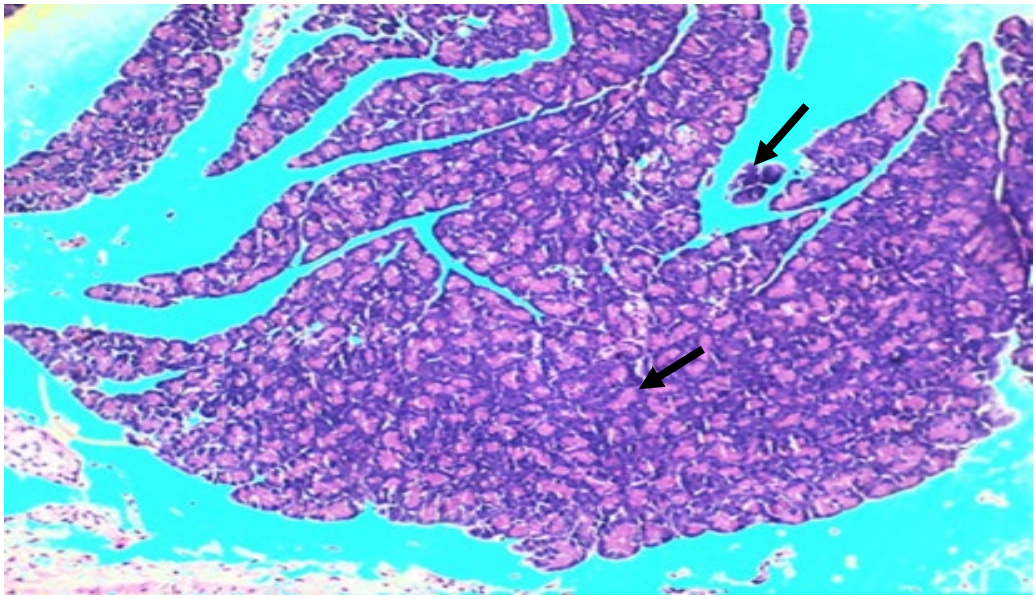


**Fig. 61. Tissue section pancreas of group 7, showing absence and atrophy of islets of Langerhans cells (H&E, X40).**

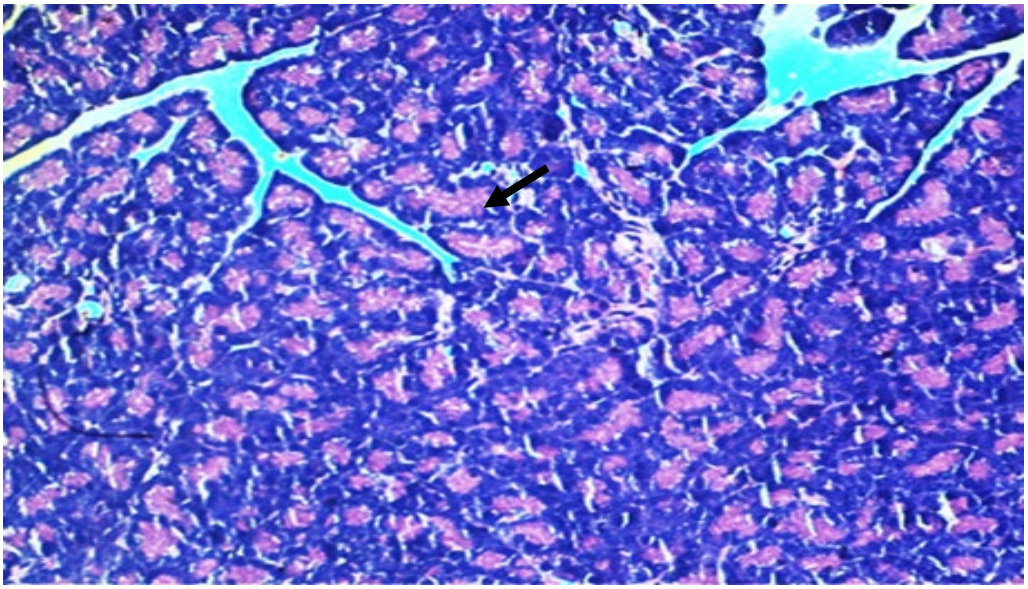


**Fig. 62. High magnification of tissue pancreas section of group 7, showing increase activating of the exocrine acini portions (H&E, X 100).**

## 4.16. Biochemical parameters of second biological experiment

### 4.16.1. Blood glucose levels

Blood glucose levels of STZ-induced diabetic groups are shown in table (21), there were induced-STZ treatment groups on fasting blood glucose, by dose STZ (65 mg/kg b.wt) and its components inhibited the destruction of rat pancreatic islet β-cells, compared with control, diabetic and medicine groups. Fasting blood glucose level of diabetic group recorded the highest level (440.3 mg/dl), followed by MED and MSE in a similar result recorded (386.3 and 382.5 mg/dl) respectively followed by MPE and MOE in a similar result recorded (352.6 and 3342.7 mg/dl) respectively.

**Table 21. Effects of *Moringa oleifera*, *Moringa stenopetala* and *Moringa peregrina* extracts on fasting and postprandial blood glucose levels of diabetic rats.**

| Treatment | FBG (mg/dl) | PPBG (mg/dl) |
|---|---|---|
| **Control** | $97.5^{d}$±1.5 | $87.7^{e}$±1.73 |
| **Diabetic** | $440.3^{a}$±3.79 | $412.0^{a}$±4.04 |
| **MED** | $386.3^{b}$±3.53 | $147.0^{d}$±2.33 |
| **MOE** | $342.7^{c}$±2.33 | $244.3^{c}$±2.65 |
| **MPE** | $352.6^{c}$±2.40 | $256.4^{bc}$±3.21 |
| **MSE** | $382.5^{b}$±2.03 | $267.5^{b}$±3.18 |
| **LSD (0.05)** | **5.23** | **7.19** |

**Each value in the table was obtained by calculating the average of the three experiments ± S.E. The superscript letters indicated statistically significant differences, with P <0.05.**

After 2 hrs. Postprandial blood glucose level for diabetic group recorded the highest level (412.0 mg/dl) followed by MSE and MPE in a similar result recorded (267.5 and 256.4 mg/dl) respectively.

On the other hand, there was a significant different observed on postprandial blood glucose level for MOE recorded (244.3 mg/dl) compared with MED group recorded (147.0 mg/dl) and Control group (87.7 mg/dl).

### 4.16.2. Effect of *Moringa oleifera*, *Moringa peregrina* and *Moringa stenopetala* leaves extract on ALT, AST and creatinine of diabetic rats.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities on STZ-induced diabetic groups are shown in table 22.

Diabetic group recorded the highest ALT activity followed by MSE in a similar result recorded 21.2 and 19.2 U/l, respectively, followed by MPE and MED groups in a similar result recorded 16.4 and 15.3 U/l, respectively, followed by MOE group recorded low activity compared with control group (12.1 and 8.1 U/l) respectively.

On the other hand, diabetic group showed the highest value on AST activity recorded 11.0 U/l, followed by MSE group 10.0 U/l, followed by MPE group in a similar result with MED group recorded 7.3 and 7.0 U/l, respectively.

MOE group showed a significant decrease in AST activity recorded 5.3 U/l compared with control group 4.3 U/l. This result is of special interest, which is lower than with previous studies of ALT range 35-48.83 U/l, Awodele *et al.,* 2012.

Diabetic group recorded a significant increase on creatinine levels recorded 1.42 mg/dl, followed by MOE and MSE groups recorded 0.65 and 0.63 mg/dl, respectively, followed by Control group recorded 0.51 mg/dl, followed by MED and MPE groups in a similar result recorded 0.45 and 0.42 mg/dl, respectively.

This result is of special interest which is in agreement with previous studies of creatinine range 1.26-2.27 mg/dl, Awodele *et al.,* 2012.

**Table 22. Effect of *Moringa oleifera*, *Moringa peregrina* and *Moringa stenopetala* leaves extract on ALT, AST and creatinine of diabetic rats.**

| Treatment | ALT* (U/l) | AST* (U/l) | Creatinine (mg/dl) |
|---|---|---|---|
| **Control** | $8.1^{d}\pm2.0$ | $4.3^{e}\pm0.21$ | $0.51^{c}\pm0.04$ |
| **Diabetic** | $21.2^{a}\pm1.13$ | $11.0^{a}\pm0.32$ | $1.42^{a}\pm0.05$ |
| **MED** | $15.3^{b}\pm0.81$ | $7.0^{c}\pm0.18$ | $0.45^{d}\pm0.02$ |
| **MOE** | $12.1^{c}\pm0.41$ | $5.3^{d}\pm0.26$ | $0.65^{b}\pm0.02$ |
| **MPE** | $16.4^{b}\pm0.18$ | $7.3^{c}\pm0.26$ | $0.42^{d}\pm0.02$ |
| **MSE** | $19.2.^{a}\pm0.43$ | $10.0^{b}\pm0.35$ | $0.63^{b}\pm0.20$ |
| **LSD (0.05)** | **6.25** | **2.69** | **0.22** |

**Each value in the table was obtained by calculating the average of the three experiments ± S.E. The superscript letters indicated statistically significant differences, with P <0.05. *Normal values: ALT (3-36 U/l); AST (0-35 U/l); Creatinine (0.7-1.4 mg/dl).**

### 4.16.3. Effect of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves extract on triglycerides, total cholesterol, HDL and LDL of diabetic rats.

The data presented in Table (23) showed that; Triglyceride level was evaluated and there was a significant different, Diabetic group recorded the highest level on Triglyceride recorded (268.3 mg/dl) followed by MSE and MPE groups in a similar result recorded (183.7 and 171.2 mg/dl) respectively,

followed by MOE and MED groups in a similar result recorded (154.0 and 151.7 mg/dl) respectively, compared with the Control group (95.3 mg/dl).

Total Cholesterol level was evaluated, Diabetic group recorded the highest level (262.8 mg/dl), followed by MSE and MPE in a similar result recorded (240.2 and 231.3 mg/dl) respectively, followed by MED group in a similar result with control group recorded (221.2 and 222.7 mg/dl) respectively. On the other hand, MOE group recorded the lowest significant decrease on Total Cholesterol level (145 mg/dl).

HDL level was evaluated; all groups recorded a significant increase compared with Diabetic group (45.1 mg/dl). MED group recorded the highest level (107.8 mg/dl), followed by MSE in a similar result with Control group recorded (89.2 and 87.2 mg/dl) respectively, followed by MPE in a similar result with MOE group recorded (77.3 and 76.7 mg/dl) respectively.

LDL level was evaluated, all groups recorded a significant decrease compared with Diabetic group recorded the highest level (165.7 mg/dl), followed by MPE and MSE in a similar result compared with Control group recorded (119.4, 114.2 and 118.2 mg/dl) respectively.

On the other hand, MED group recorded a significant decrease (93.0 mg/dl), while MOE group recorded the lowest level on LDL (38.2 mg/dl). *Moringa oleifera* leaves powder ethanolic extract (450 mg/kg body weight) reduced the (plasma) blood glucose level on diabetic rats to (244.3 mg/dl). *Moringa oleifera* leaves powder aqueous extract (225 mg/kg body weight) reduced and improved liver function activity (ALT) to (6.2 U/l). *Moringa*

*oleifera* leaves powder ethanolic (600 mg/kg body weight) reduced and improved liver function activity (AST) to (4.3 U/l).

*Moringa oleifera* leaves powder ethanolic extract (300 and 450 mg/kg body weight) reduced and improved kidney function activity (creatinine) to (0.65 U/l). *Moringa oleifera* leaves powder ethanolic extract (450 and 600 mg/kg body weight) and aqueous extract (190 mg/kg body weight) reduced and improved triglyceride level to (154 and 161.2, and 164.7 mg/dl) respectively.

**Table 23. Effect of *Moringa oleifera*, *Moringa peregrina* and *Moringa stenopetala* leaves extract on triglycerides, total cholesterol, HDL, and LDL of diabetic rats.**

| Treatment | Triglycerides* (mg/dl) | Total* Cholesterol (mg/dl) | HDL* (mg/dl) | LDL* (mg/dl) |
|---|---|---|---|---|
| **Control** | 95.3 $^{d}$±1.16 | 222.7$^{bc}$±2.26 | 87.2$^{b}$±1.45 | 118.2$^{b}$±1.46 |
| **Diabetic** | 268.3$^{a}$±3.21 | 262.8$^{a}$±2.07 | 45.1$^{d}$±1.40 | 165.7$^{a}$±2.31 |
| **MED** | 151.7$^{c}$±1.76 | 221.2$^{c}$±2.02 | 107.8$^{a}$±1.75 | 93.0$^{c}$±2.08 |
| **MOE** | 154.0$^{c}$±2.65 | 145.7$^{d}$±2.04 | 76.7$^{c}$±1.08 | 38.2$^{d}$±2.03 |
| **MPE** | 171.2$^{b}$±2.03 | 231.3$^{bc}$±2.67 | 77.3$^{c}$±0.87 | 119.4$^{b}$±1.79 |
| **MSE** | 183.7$^{b}$±1.20 | 240.2$^{b}$±2.32 | 89.2$^{b}$±1.16 | 114.2$^{b}$±1.83 |
| **LSD(0.05)** | **4.87** | **5.65** | **3.42** | **6.53** |

**Each value in the table was obtained by calculating the average of the three experiments ±S.E. The superscript letters indicated statistically significant differences, with P <0.05. *Normal values: Triglycerides (<195 mg/dl); Total Cholesterol (<200 mg/dl); HDL (<40 mg/dl); LDL (<77.3 mg/dl)**

*Moringa oleifera* leaves powder ethanolic extract (450 mg/kg body weight) reduced and improved the total cholesterol level (200 mg/dl). *Moringa oleifera* leaves powder aqueous extract (190 mg/kg body weight) reduced and improved the high-density cholesterol level (47.2 mg/dl). All *Moringa* leaves powder aqueous and ethanolic extracts reduced and improved the low-density cholesterol level (77 mg/dl).

In the present study, the injection of STZ induces hepatocellular damage, which is one of the characteristic changes in diabetes as evidenced by high serum levels of AST and ALT in untreated diabetic control group (2). The release of these enzymes into the serum as a result of tissue injury or changes in the permeability of liver membranes; hence the concentration may increase with acute damage to liver cells, (Hsueh *et al.,* 2011).

When the integrity of the hepatocellular membrane is compromised, there is extrusion of the marker enzymes into the plasma, (Moss *et al.,* 1996). Therefore, the significant elevation ($P<0.05$) in the levels of the marker enzyme in the serum as observed in the diabetic untreated group 2 when compared to the normal group 1 suggests possible damage to the liver or muscle.

However, the treated groups with aqueous and ethanolic extract of *Moringa* leaves powder showed a significant reduction in the levels of these enzymes when compared to the diabetic group 2, thus an indication of the protective effects of the extracts over STZ-induced liver damage accords with the report of other investigators, (Sharma *et al.,* 2010).

The levels of the marker enzymes (ALT and AST) particularly in the ethanolic extracts treated group were lower than the aqueous extracts treated group, suggesting a strong hepatoprotective ability of the ethanolic extracts. These levels are markedly reduced in the extract-treated groups, suggesting the enhancement of liver functions by the extracts. These accords with similar findings on these biochemical parameters by James *et al.*, (2014), Usha *et al.,* (2008), Atawodi *et al.,* 2010 and Ladeji and Okoye, (1996). There was significant decrease (P>0.05) in the serum activities of the marker enzymes (ALT and AST) in the normal group rats, thus suggesting little or low level of toxicity of the extracts.

### 4.16.4. Histopathological examination for the second biological experiment

Histological changes of liver, pancreas and kidney tissues of STZ-induced diabetic rats in different groups treated with a single daily dose 450 mg/kg b.w. ethanolic extract of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves, compared with MED group treated with a single daily dose 100 mg/kg b.w. of Cedophage as traditional medicine drug for diabetes rats, in presence of normal control and diabetic groups.

From the histopathological examination of the tissues; liver, pancreas and kidneys of STZ-induced diabetic rats with different treated groups (MED, MOE, MPE and MSE) compared to both control negative and positive groups as shown in figures (63-71).

### 4.16.5. Histopathological of liver structures

For liver of diabetic rat group treated with common medicinal drug (Cedophage 50 mg) MED group, Fig. (63) showed improving liver tissues compared with diabetic group (2).

On the other hand, liver of diabetic rat group treated with ethanolic extract of *Moringa peregrina* leaves powder (450 mg/kg b.w) group (MPE), Fig. (64) showed degenerative changes of the hepatic cells and activation of Van Kupffer cells, dilatation of portal area with proliferation of newly formed bile ducts and different forms of nacrobiotic changes of hepatocytes.

However the treatment with the ethanolic extract of *Moringa stenopetala* leaves powder (450 mg/kg b.w) group (MSE), Fig. (65) showed degenerative changes of the hepatic cells and activation of Van Kupffer cells, dilatation of portal area with proliferation of newly formed bile ducts and different forms of nacrobiotic changes of hepatocytes, on the other hand, the MSE group did not show any improving of liver of diabetic rats compared with diabetic control group (2).

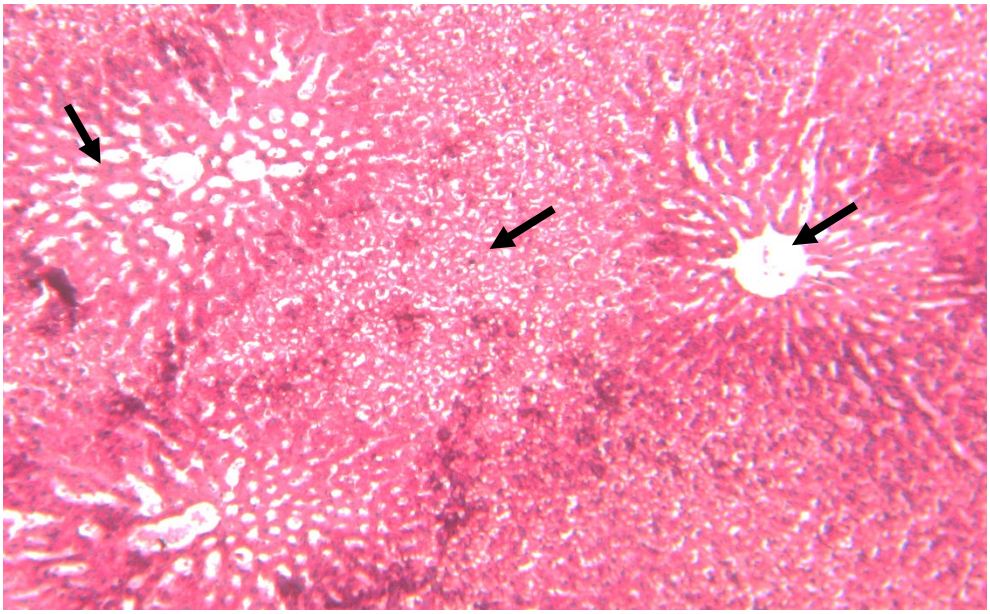


**Fig. 63. MED group, showing the treated rat with traditional medicine (Cedophage) histological structure of hepatic lobule (H&E, X 100).**

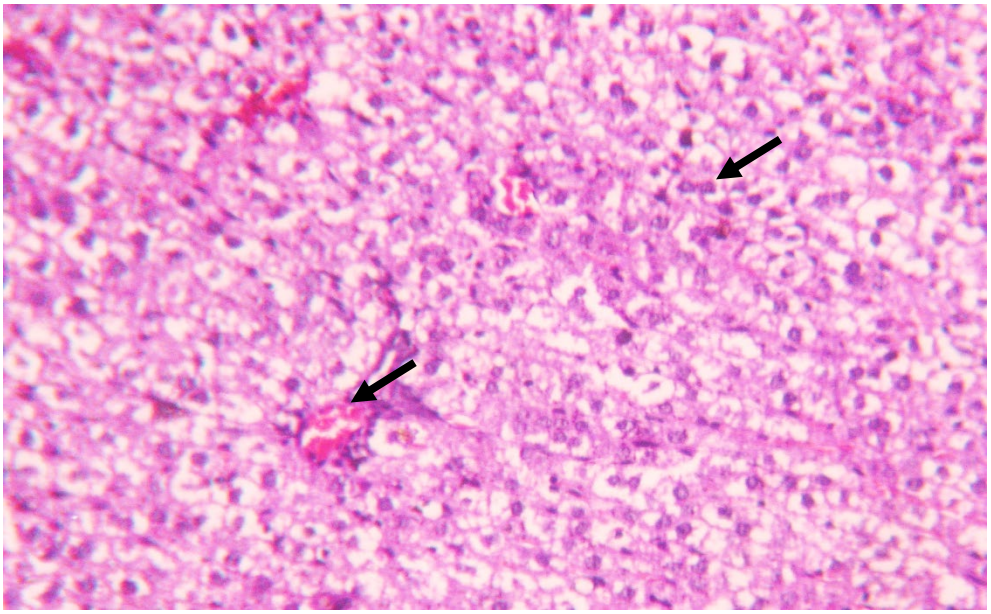


**Fig. 64. MPE group showing degenerative changes of the hepatic cells and activation of Van Kupffer cells, dilatation of portal area with**

**proliferation of newly formed bile ducts and different forms of nacrobiotic changes of hepatocytes**

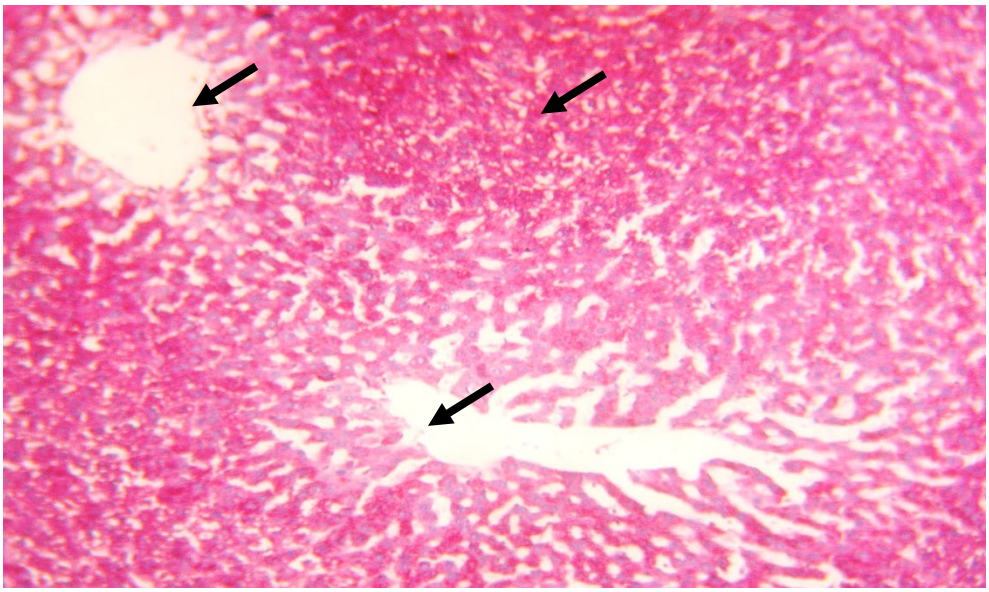


**Fig. 65. MSE group showing degenerative changes of the hepatic cells and activation of Van Kupffer cells, dilatation of portal area with proliferation of newly formed bile ducts and different forms of nacrobiotic changes of hepatocytes**

## 4.16.6. Histopathological of kidney structures

For kidneys tissues, the histological of MED group Fig. (66) showing renewing structure of renal tissue treated by a traditional medicinal drug (Cedophage), renal tubules.

On the other hand, renal tissues of MPE group, Fig. (67) showing degenerative changes of renal glomeruli and degenerative changes of epithelial cell of renal tubules.

Last, renal tissue of MSE group, Fig. (68) showing advanced degeneration of renal tubules and damage of most bowman capsules and atrophy and disintegration of the glomerular tuft.

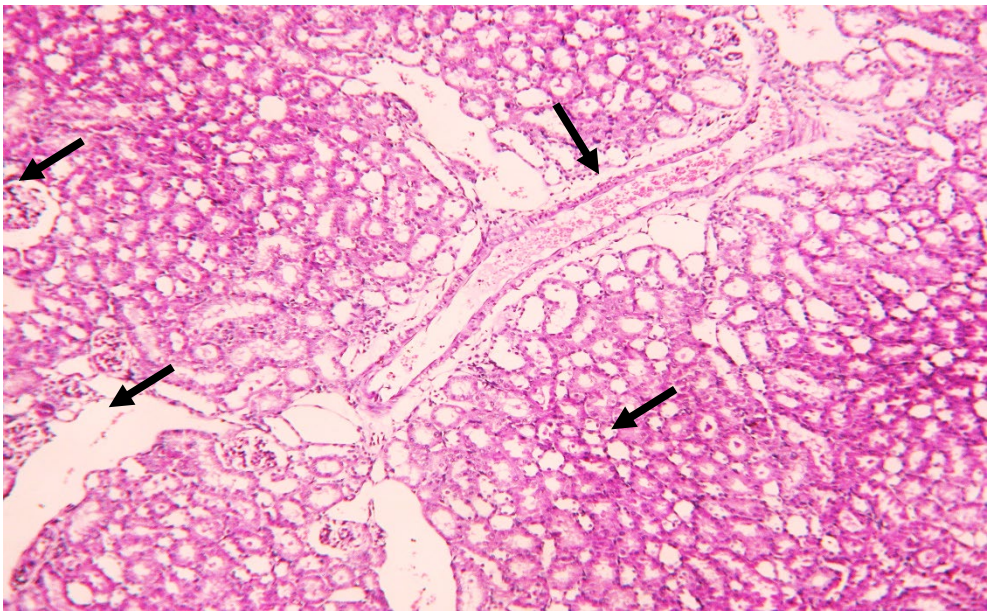


**Fig. 66. Renal tissue of MED group, showing renewing structure of renal tissue treated by a traditional medicinal drug (Cedophage), renal tubules (H &E, X100).**

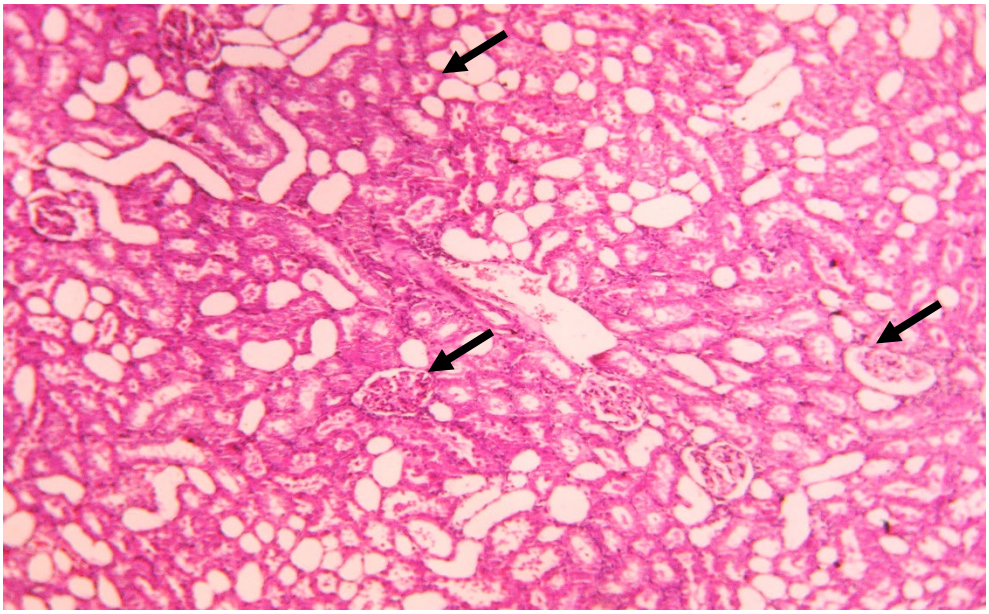


**Fig. 67. Renal tissue of MPE group, showing degenerative changes of renal glomeruli and degenerative changes of epithelial cell of renal tubules (H &E, X100).**

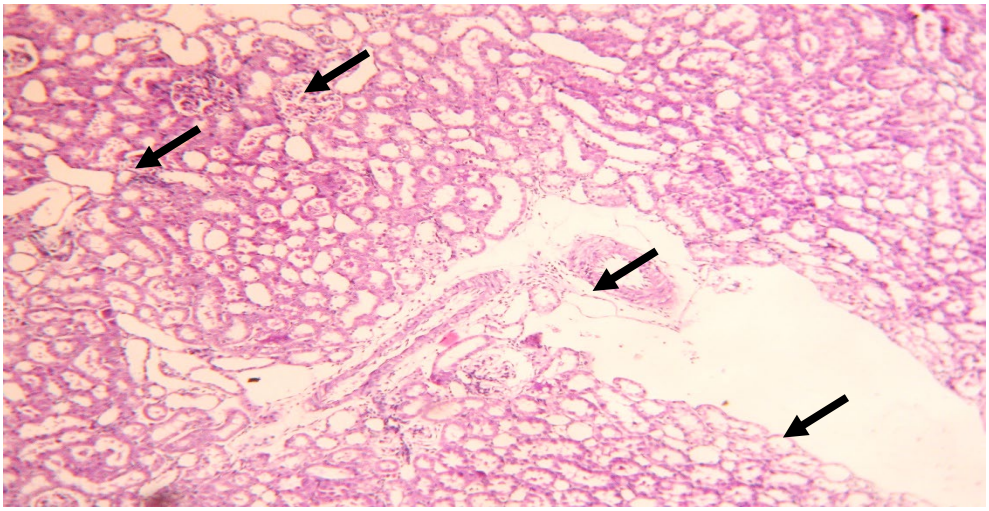


**Fig. 68. Renal tissue of MSE group, showing advanced degeneration of renal tubules and damage of most bowman capsules and atrophy and disintegration of the glomerular tuft (H&E, 200).**

### 4.16.7. Histopathological of pancreas structures

For pancreatic tissues, MED group of tissue pancreas section, Fig. (69) showing increase activating of the exocrine acini portions, also presence of islets of Langerhans may be similar to normal group (1). MPE group of tissue section pancreas, Fig. (70) showing, very few foci of islets of Langerhans, the cells showing degenerative changes. MSE group tissue section pancreas, Fig. (71) showing degenerative an atrophy of islets of Langerhans cells. While MPE group, showed slightly improving of hepatocytes, kidney tissue with atrophy and degeneration of islets of Langerhans compared with diabetic control group (2).

On the other hand, the MSE group did not show any improving of liver, pancreas and kidneys of diabetic rats compared with diabetic control group (2). The ethanolic extract of *Moringa oleifera* leaves powder (450 mg/kg body weight) improved and maintained the form and syntax of the liver cells and stop the bloodshed of the cells, not cystic cells, also contributed to the improvement in liver and kidney structures and the restoration of activity of the remaining injury pancreatic cells, there was a sign of regeneration of the islet, which accord with other publications, (Xiu *et al.,* 2001). However the treatment with the ethanolic extract of *Moringa* leaves powder used, have slight toxic to hepatocytes. Liver is known to be the main target of detoxification to a wide variety of toxic compounds and / or its metabolites due to its content from the main enzyme known as cytochrome P450.

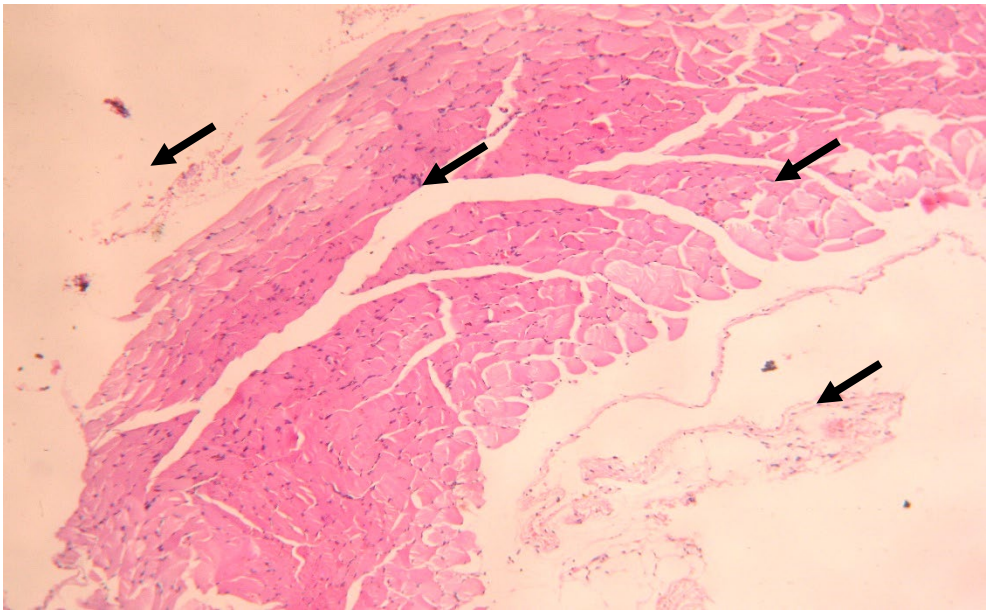


**Fig. 69. MED group of tissue pancreas section, showing increase activating of the exocrine acini portions (H&E, X 100).**

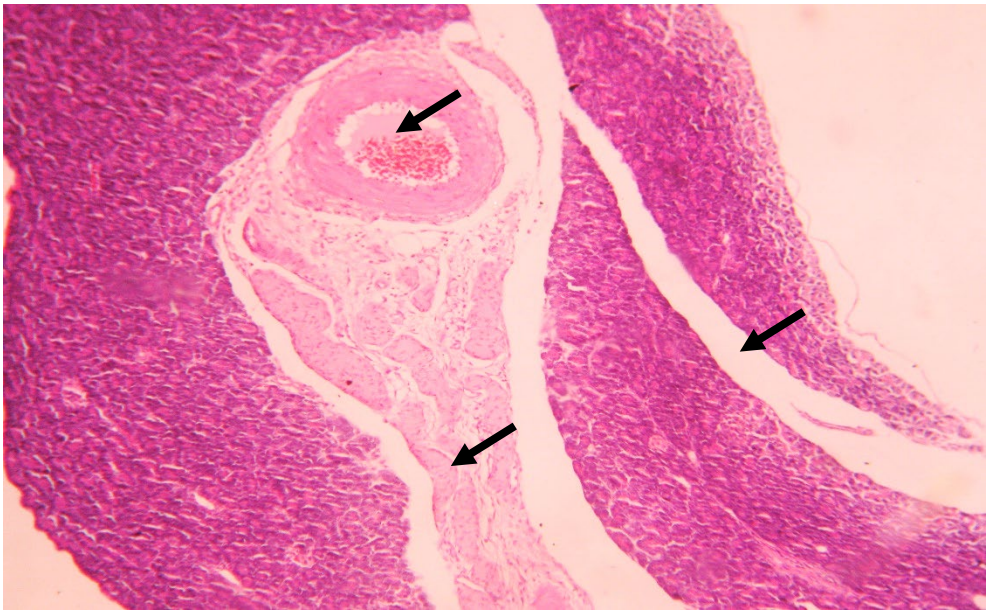


**Fig. 70. MPE group of tissue section pancreas showing, very few foci of islets of Langerhans, the cells showing degenerative changes. H&E, X100).**

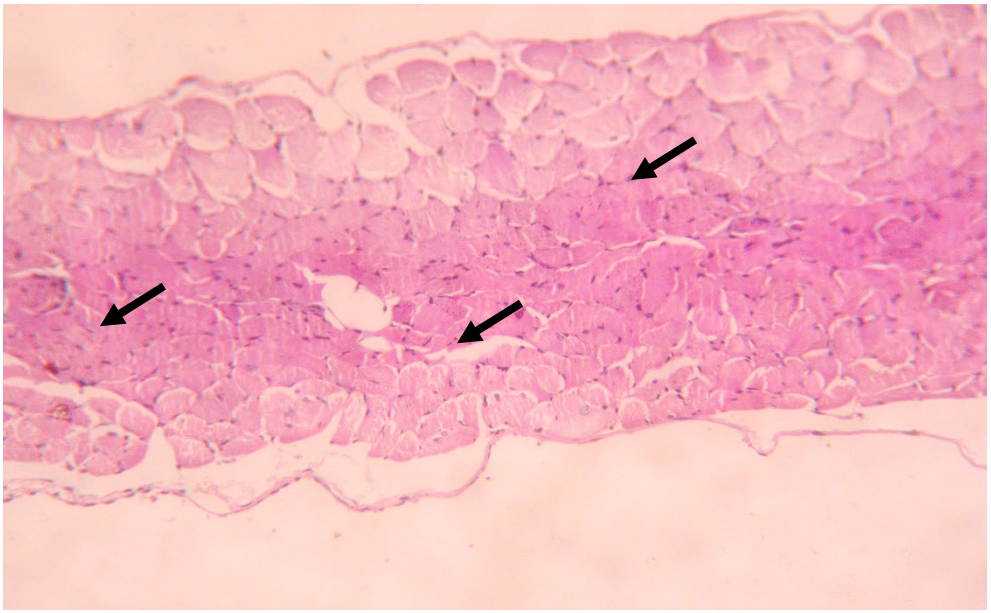


**Fig. 71. MSE group tissue section pancreas, showing degenerative an atrophy of islets of Langerhans cells (H&E, X100).**

The cytochrome P450 superfamily of monooxygenases (CYP) is a large and diverse group of enzymes that catalyze the oxidation of organic substances. The substrates of CYP enzymes include metabolic intermediates such as lipids and steroidal hormones, as well as xenobiotic substances such as drugs and other toxic chemicals. CYPs are the major enzymes involved in drug metabolism and bio activation, accounting for about 75% of the total number of different metabolic reactions (Guengerich, 2008).

In this study, ethanolic extract of *Moringa* leaves powder showed significant decrease in blood glucose level of diabetic rats by STZ induction in agreement with the reports of *Moringa* leaves extract, which has been shown to be effective in lowering blood sugar levels within 3 hrs. ingestion,

though less effectively than the standard hypoglycemic drug glibenclamide, (Manjari *et al.,* 2007).

The antidiabetic effect of the *Moringa* extract was further concluded by the histopathological examinations. The results showed increase of improved liver tissues compared with diabetic control group, however the treatment with used extract has slight toxic to hepatocytes, kidneys and pancreatic tissues presence of islets of Langerhans may be similar to control group (1). The *Moringa* extracts at different dose levels offer hepatoprotection, especially group (4) treated with MOL (450 mg/kg b.w) was more effective than all other groups and this may be due to its antidiabetic effect and produced a good defense mechanisms to prevent the formation of these tissues. Reports have shown that the reduction in pancreatic β-cells can be as high as 50% during diabetes Zhou *et al.,* (2012), Wang *et al.,* (2010) and Lee *et al.,* (2010).

The present study revealed the presence of damaged β-cell population due to STZ induction. The histopathological study of diabetes-treated groups indicates increase in the volume density of islets, percent of β cells and size of islet in the groups that received the plant extracts, which may be a sign of regeneration along with β-cells repairs. Administration of the extract to non-diabetic rats show distorted pancreatic architecture and mononuclear cellular infiltration and sinusoidal spaces, suggesting that the extracts at the concentration used is relatively toxic to the pancreas of non-diabetic rats. These reports were consistent with the present study, thus islet cell

replacement or regeneration therapy may offer therapeutic benefit to people with diabetes, in agreement with Moss *et al.,* (2009) and Halban *et al.,* (2010). Photo micro graphical data in this study suggests that the healing of pancreas by ethanolic extracts of *Moringa* leaves may be a plausible mechanism of their anti-diabetic activity. The data obtained showed the ability of *Moringa* extract to stabilize the liver marker enzymes for diabetes i.e. reduces the elevated level of these marker enzymes (ALT and AST) which were caused by the diabetes. This action is in agreement with other medicinal plant extracts that are known antidiabetic agents, (Sharma *et al.,* 2010).

This study, clearly showed that *Moringa* is not just an antidiabetic agent but has the ability to regenerate damaged pancreatic cells. It adds further credence to the use of the plant as an antidiabetic agent and to the ability of medicinal plants to regenerate islet of Langerhans. Also, showed that *Moringa* leaves can be used for the management of diabetes as well as regenerate damaged pancreatic islets. In addition, the variation in the heavy metal contents of *Moringa* leaves found in this study when compared to earlier studies may be due to climatic and edaphic factors, solvents used for the analysis, the cultivation method used and age of the plant.

Furthermore, the leaves have been known to serve as a herbal tonic and for the maintenance of tissues and cell membranes. Many herbs and plants have been described as possessing hypoglycemic activity when taken orally. Some of these plants have also been pharmacologically tested and shown to be of some value in human type II diabetes mellitus treatment. The

efficacy of herbal drugs is significant and they have fewer side effects than the synthetic allopathic medicines. Further, phytochemical characterization of medicinal plants is required to identify the specific compound (s) involved in the observed hypoglycemic.

## 4.17. Study recommendations and conclusions

The multiple benefits of *Moringa* made it a true miracle of nature. *Moringa oleifera* plant is the most inexpensive and credible alternative to not only providing good nutrition, but also the cure and prevention of many diseases.

1. From the results of this study, it is recommended using of *Moringa oleifera* leaves powdered, ethanolic and aqueous extract (450 mg/kg body weight) daily is safely for patients with diabetes to improve blood glucose level, as well as to improve liver, kidney and pancreas functions.
2. Use and eating extract or powder of *Moringa oleifera* leaves for children, pregnant, lactating women and all people ages as it contains high occasion of calcium, iron, magnesium , zinc and copper elements values, because they contain vitamin (A) and vitamin (C), essential amino acids and high in protein (28% ), the high content of natural antioxidants, high total phenolic compounds content, as well as the high content of functional groups necessary as secondary metabolites.
3. Use and eat *Moringa* seed oil types under study for the content of Omega (3) and Omega (6) content, the poly unsaturated fatty acids and the lack of content of saturated fatty acids.

4. Industrial uses of *Moringa* seed oil types under consideration for the low percentage of free fatty acids, low peroxide value, iodine number and saponification value.
5. Non-use *Moringa* seed oil types under study in the solo for frying.

# 5. UMMARY

*Moringa oleifera, Moringa stenopetala, Moringa peregrina* belong to a family *Moringaceae*. The aim of this investigation was to determine and evaluate (*in vitro*) the chemical composition of leaves and seeds contents for *Moringa stenopetala, Moringa oleifera, and Moringa peregrina* which were collected from five places under Egyptian conditions.

Fresh *Moringa stenopetala* leaves and seeds were collected from Aswan botanic garden, and Belbis, Sharkya, Egypt. Fresh *Moringa oleifera* leaves and seeds were collected from Model Plant Farm of Nubarya and Shalateen, Egypt. Fresh *Moringa peregrina* leaves and seeds were collected from Orman botanic garden, Giza, Egypt. The collected samples were purified and allowed to air and sun drying, leaves and seeds were manually ground in mortar.

The experiments and analysis were carried out in 2014 and 2015 at the regional center for food and feed laboratories, for the fatty acids, amino acids, elements, vitamins, protein, ash, fat, energy, mass spectrum analysis's and biological experiments. In addition to evaluate the effects of ethanolic and aqueous extract of *Moringa oleifera* leaves (MOL) on streptozotocin (STZ)-induced diabetes rats.

## 5.1 Proximal analysis of leaves and seeds for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

The results of the proximate composition in this study; *Moringa* has high nutritional values; seeds contain large amounts of protein (23.5-30.0 g/100 g dry weight), MPG and MON recorded the heights values of protein (30±0.1%, 29.9±0.1%) respectively. The seeds also contain high amount of fiber (21.8 to 30.7%) and significant amounts of ash (5.1 to 7.6%). The leaves also contain high amount of fiber (8.8 to 16.5%) and significant amounts of ash (8.1 to 10.5%).

## 5.2 Seeds and leaves energy for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

*Moringa* has high nutritional values; seeds and leaves contain large amounts of energy (3839 to 6623 kcal/kg dry weight), there were significant different *M. peregrina* seeds (MPG) recorded the highest value, but its leaves recorded the lowest value (3839, 6623 kcal/kg) respectively. On the other hand, *M. stenopetala* leaves (MSB) recorded the heights value of Energy (4214 kcal/kg).

On the other hand, balance in the results of Energy for leaves and seeds, the value of energy for seed is high but the value of energy for leave were low. That balances and different may referred to many factors; environments, climate, origin, genetic, fertilization and temperature range.

## 5.3 Elements analysis for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves and seeds.

*Moringa* has high nutritional value, leaves and seeds contain large amounts of Ca range as recorded (181.95-1529 ppm dry weight) for leaves

and (520.8-1994 ppm) for seeds, MON recorded a significant increase amount of Ca (1994 ppm) for seeds and MOS recorded a significant increase amount of Ca (1529 ppm) for leaves. Narrow range of Mg values were recorded (142-169 ppm) for leaves and (124-154 ppm) for seeds. On the other hand, Fe recorded range (3.67-44.55 ppm) for leaves and (25.17-62.45 ppm) for seeds. Zn recorded range (0.0029-0.1781 ppm) for leaves and (0.0011-.0048 ppm) for seeds. Cu recorded range (0.09-0.805 ppm) for leaves and (0.0-1.07 ppm) for seeds.

## 5.4. Free fatty acid percentage, peroxide, iodine and saponification values of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* seeds oil.

*Moringa* seeds contain large amounts of oil, free fatty acids percentage recorded narrow range (0.32-0.8%). Peroxide values recorded a significant different and range (2.27- 5.03 mEq. $O_2$/kg Oil). Iodine values recorded similar results and narrow range (65.34-70.07). On the other hand, Saponification values recorded similar results and narrow range (175.7-192.3 mEq. KOH/kg Oil)

## 5.5. Fatty acids composition of seeds for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

The fatty acids compositions for *Moringa* oil were recorded. The major saturated fatty acids were palmitic (C16:0) acids, stearic (C18:0) and behenic (C22:0) ranged from (5.93-10.31), (3.49-7.26) and (2.56-5.94)respectively. The main unsaturated fatty acid is oleic acid (C18:1w9) with small amounts eicosenoic (C20:1w9) and palmitoleic acids (C16:1w7).

## 5.6. Amino acids composition of leaves for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala*

The Amino Acids compositions for *Moringa* leaves and seeds recorded significant increase for total summation of amino acids percentage (9.85-24.87%). On the other hand, Amino Acid/Protein Ratio recorded high percentage range (80.08-85.45%).

## 5.7. Total phenolic contents and antioxidants scavenging activity on DPPH radicals for *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves.

The total phenolic contents and antioxidants scavenging activity on DPPH radicals were determined, for ethanolic extracts of dried leaves of *Moringa* species from different agro climatic regions. The total phenolic contents recorded significant increases, (23.3-38.3 mg/g GAE), MPG recorded the highest value 38.3 mg/g GAE. On the other hand, antioxidants scavenging activity on DPPH radicals recorded (42.82-62.11%), the ethanolic extracts of MSA leaves showed the highest antioxidant activities, 62.11% .

## 5.8. Experimental biology

Acute STZ-induced diabetic albino rats under studies were treated with aqueous and ethanolic extract of *Moringa oleifera* leaves for a period of (30) days for diabetic study. The diabetic rats revealed nontoxic nature of the *Moringa oleifera* at a concentration of (600 mg/kg of body weight/day) for this period.

There were some morphological changes like weight losses, more drinking water and more urine. There was no lethality or any toxic reactions found at either doses selected till the end of treatment period.

The biological experiment was divided into two stages; in the first stage 42 male albino rats were divided into 7 groups. Group1; normal control, group 2; diabetic control, diabetic groups 3, 4 and 5 treated with MOL ethanolic extracts 600, 450 and 300 mg/kg b.wt, respectively and diabetic groups 6 and 7 were treated with aqueous 190 and 225 mg/kg b.wt respectively. Fasting (FBG) and postprandial blood glucose (PBG) levels, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Creatinine, Triglyceride (TG), Total Cholesterol (TC), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) were evaluated.

Depending on the pervious experiment stage result, 450 mg/kg b.w of MS and MP ethanolic leaves powder were used on STZ-induced male albino rats groups, in addition, MED group was treated with cedophage 100 mg/kg b.w, as a traditional medicine group compared with MO, diabetic and normal control groups.

## 5.9. Blood glucose levels

Blood glucose levels of STZ-induced diabetic groups were measured, there was no significant different among induced-STZ treatment groups on fasting blood glucose, that mean good affecting of that dose of induced STZ (65 mg/kg b.wt)

On the other hand, while glucose level postprandial 2 hrs. was significantly high in diabetic control group, the level of blood glucose was significantly decreased in groups 4, 5 and 7 compared with diabetic group 2 (244.3, 274.7, 249.3 and 420.0 mg/dl) respectively.

In addition, there were significant different observed on postprandial 4 hrs. blood glucose levels among groups 4, 5 and 7 compared with diabetic group 2 (278.7, 283.3, 251.0 and 407.3 mg/dl) respectively, the same results were observed on postprandial 6 hrs. blood glucose levels among groups 4 and 7 compared with diabetic group 2 (277.3, 284.7 and 427.3 mg/dl) respectively.

## 5.10. Effect of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves extract on alanine aminotransferase, aspartate aminotransferase and creatinine of diabetic rats.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities on STZ-induced diabetic groups. Groups 3, 4, 6 and 7 showed significant decrease compared with diabetic group 2, (13.5, 12.1, 13.9, 6.2 and 19.4 U/l), respectively.

Also significant decrease on (AST) activity were observed for groups 3, 4, 5, 6 and 7 compared with diabetic group 2 (6.3, 5.3, 4.3, 7.3, 6.0 and 9.0 U/l) respectively.

Creatinine levels recorded significant decrease for groups 3, 4, 5, 6 and 7 compared with diabetic group 2 (0.65, 0.65, 1.04, 0.81, 0.90 and 1.23 mg/dl)

respectively, also in a nearly result when compare with control group 1 recorded (0.43 mg/dl).

## 5.11. Effect of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves extract on triglycerides, total cholesterol, HDL and LDL of diabetic rats.

Triglyceride level was evaluated and there was significant decrease for groups 3, 4, 5 and 6 compared with diabetic group 2 (242.7, 154.0, 161.2, 164.7 and271.3 mg/dl) respectively. Total Cholesterol level was recorded significant decrease for groups 3, 4, 5, 6 and 7 compared with diabetic control group 2 (154.3, 145.7, 160.9, 140.5, 167.7 and 259.8 mg/dl) respectively.

Significant increase on HDL level was evaluated for groups 3, 4, 5 and 7 compared with control group 2 (65.8, 76.7, 59.3, 47.2, 73.3 and 42.1 mg/dl) respectively. Group 6 recorded significant decrease of HDL level, in a similar result of diabetic control group 2 (47.2 and 42.1 mg/dl) respectively. Significant decrease on LDL level was recorded for groups 3, 4, 5, 6 and 7 compared with control group 2 (40.0, 38.2, 69.4, 60.4, 43.1 and 163.5 mg/dl) respectively.

## 5.12. Histopathological examination

Histological changes of liver, pancreas and kidney tissues of STZ-induced diabetic rats in different groups treated with ethanolic and aqueous extract of MOL were studied. The histopathological examination of the tissues; liver, pancreas and kidneys of STZ-induced diabetic rats with different treated groups group 3, 4, 5, 6 and 7 compared to both control negative and positive groups.

Group 4 showed increase of improved liver tissues compared with diabetic control group, however the treatment with the extract used have slight toxic to hepatocytes kidneys tissues and pancreatic tissues also presence of islets of Langerhans may be similar to group 1, While group 5, showed slightly improving of hepatocytes, kidney tissue with atrophy and degeneration of islets of Langerhans when compared with diabetic control group 2. On the other hand, the groups 3, 6 and 7 did not show any improving of liver, pancreas and kidneys of diabetic rats compared with diabetic control group 2.

## 5.13. Histopathological examination for the second biological experiment

Histological changes of liver, pancreas and kidney tissues of STZ-induced diabetic rats in different groups treated with a single daily dose 450 mg/kg b.w. ethanolic extract of *Moringa oleifera, Moringa peregrina* and *Moringa stenopetala* leaves, compared with MED group treated with a single daily dose (Cedophage 100 mg/kg b.w) as traditional medicine drug for diabetes rats, in presence of normal control and diabetic groups.

From the histopathological examination of the tissues; liver, pancreas and kidneys of STZ-induced diabetic rats with different treated groups (MED, MOE, MPE and MSE) compared to both normal and diabetic control groups.

For liver of diabetic rat group treated with common medicinal drug (Cedophage 100 mg/kg b.w) MED group showed improving liver tissues compared with diabetic group (2).

However the treatment with the ethanolic extract of *Moringa* leaves powder used, have slight toxic to hepatocytes.

On the other hand, group (MPE) showed degenerative changes of the hepatic cells and activation of Van Kupffer cells, dilatation of portal area with proliferation of newly formed bile ducts and different forms of nacrobiotic changes of hepatocytes.

For kidneys tissues, the histological of MED group showing renewing structure of renal tissue treated by a traditional medicinal drug (Cedophage), renal tubules. On the other hand, MPE group, showing degenerative changes of renal glomeruli and degenerative changes of epithelial cell of renal tubules. MSE group, showed advanced degeneration of renal tubules and damage of most bowman capsules and atrophy and disintegration of the glomerular tuft.

For pancreatic tissues, MED group showed increase activating of the exocrine acini portions, also presence of islets of Langerhans may be similar to normal group (1). MPE group of tissue section pancreas showed very few foci of islets of Langerhans, the cells showing degenerative changes, while MSE group degenerative an atrophy of islets of Langerhans cells.

While MPE group, showed slightly improving of hepatocytes, kidney tissue with atrophy and degeneration of islets of Langerhans compared with diabetic control group (2). On the other hand, the MSE group did not show any improving of liver, pancreas and kidneys of diabetic rats compared with diabetic control group (2).

The ethanolic extract of *Moringa oleifera* leaves powder (450 mg/kg body weight) improved and maintained the form and syntax of the liver cells and stop the bloodshed of the cells, not cystic cells, also contributed to the improvement in liver and kidney structures and the restoration of activity of the remaining injury pancreatic cells.

Result showed high significant decrease for PBG, creatinine, TC, TG, HDL and LDL levels, ALT, AST activities. Meanwhile, histopathological examination for liver, kidney and pancreas tissues showed more improving for MO group than the other groups.

## 5.14. Study recommendations

From the above results of this study recommends that:

1- Using ethanolic or aqueous extract (450 mg / kg body weight ) of powdered leaves *Moringa oleifera* , a day for patients with diabetes to improve blood glucose level , as well as to improve liver, kidney , pancreas and functions without the slightest damage.

2- Use extract or powder of *Moringa oleifera* leaves for human nutrition because it contains high occasion of calcium, iron, magnesium, zinc and copper elements values, because they contain vitamin (A) and vitamin (C), essential amino acids and high in protein (28%), and the high content of natural antioxidants, and total phenolic compounds content, as well as the high content of necessary functional groups.

3- Use all *Moringa* seed oil types under study for the content of omega 3 and omega 6 content and many of the poly unsaturated fatty acids and the lack of content of saturated fatty acids.

4- Use and eat all *Moringa* seed oil types under consideration for the low percentage of free fatty acids , low peroxide value and low iodine number and low saponification value.

5- Non-use of all *Moringa* seed oil types under study in the solo for frying.

# 6. REFFERENCES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# AFRICAN STUDIES

(Natural Resources-Plant Resources)

**"Agric. Biochemistry"**

THESIS
Submitted in Partial Fulfillment of the
Requirements for the Degree of

## DOCTOR OF PHILOSOPHY

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